PHYSIOLOGICAL PARAMETERS OF SALINITY TOLERANCE IN C_4 TURFGRASSES

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ABSTRACT

Growth and physiological responses to salinity of 13 C_4 turfgrasses, and of a C_4 coastal salt marsh grass, were compared in an attempt to elucidate salinity tolerance mechanisms operating in these grasses. Relative shoot growth reduction with increasing salinity, as a percent of control, and also visual quality ratings, were used as indicators of salt tolerance. *Sporobolus virginicus* (L.) Kunth (a coastal salt marsh grass), seashore paspalum (*Stenotaphrum secondatum* Walt.), and Manilagrass (*Zoysia matrella* L.) consistantly ranked as the most salt tolerant. The bermudagrasses (*Cynodon spp.* (L.) Pers., Burtt-Davey) were intermediate in salt tolerance, though the common bermudagrass Hawaii selections were more tolerant than the other bermudagrasses studied. Japanese lawngrass (*Zoysia japonica* Steud.) was salt sensitive, and centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.) very salt sensitive, respectively. Root growth was stimulated by intermediate salinities in the burmudagrasses, seashore paspalum, and Manilagrass, and by high salinities in *S. virginicus*. Increased root growth may result in more effecient water and nutrient uptake under salinity stress. All grasses adjusted osmotically, maintaining sap osmolalities above that of salinity media. Though shoot Na^+ and Cl^- accumulation was primarily responsible for osmotic adjustment, shoot dehydration also contributed. In grasses other than St. Augustinegrass, salinity tolerance was associated with exclusion of Na^+ and Cl^- from shoots, coupled with shoot selectivity for K^+ over Na^+. Seashore paspalum relied on selective ion uptake/exchange mechanism of the root

cortex, while in *S. virginicus*, bermudagrasses, and manilagrass shoot Na^+ and Cl^- exclusion was aided by very active leaf salt glands. Shoot Na^+ and Cl^- concentrations reached high levels in Japanese lawngrass and centipedegrass, resulting in shoot death at relatively low salinities. Growth stimulation at intermediate salinity, associated with ion accumulation and increased shoot succulence, typical halophytic responses, occured in *S. virginicus* and St. Augustinegrass. St. Augustinegrass accumulated Na^+ and Cl^- to much higher levels than other grasses, and maintained very high shoot succulence. Shoot ion concentrations were sufficiently high in all grasses at high external salinity to necessitate ion compartmentation in vacuoles. Proposed compatible solutes glycinebetaine and proline accumulated sufficiently to make substantial contributions to cytoplasmic osmotic adjustment in all grasses except centipedegrass.

TABLE OF CONTENTS

ABSTRACT.....iii

LIST OF TABLES.....vii

LIST OF FIGURES.....xi

CHAPTER I. INTRODUCTION.....1

CHAPTER II. LITERATURE REVIEW.....3

Salinity Tolerance in Turfgrasses.....3
Morphological Adaptations to Salinity.....5
Osmotic Relations.....6
Ion Uptake/Exclusion.....8
Intracellular Solute Compartmentation.....15
Compatible Solutes.....18
i) Glycinebetaine.....18
ii) Proline.....19
iii) Evidence of Location in Cytoplasm.....20
iv) Evidence-Enzyme Protection.....20

CHAPTER III. GROWTH RESPONSES, ION RELATIONS, AND OSMOTIC ADAPTATIONS OF ELEVEN C_4 TURFGRASSES TO SALINITY.....23

ABSTRACT.....23
INTRODUCTION.....24
MATERIALS AND METHODS.....25
RESULTS AND DISCUSSION.....29
Growth Responses.....29
Tissue Ion Concentrations.....32
Osmotic And Water Relations.....37

CHAPTER IV. SALT GLANDS IN THE ZOYSIEAE.....43

ABSTRACT.....43
INTRODUCTION.....43
MATERIALS AND METHODS.....45
Growing Conditions.....45
Ion Contents.....45
Sap Osmolality.....46
Electron Microscopy.....46
RESULTS AND DISCUSSION.....47

CHAPTER V. GROWTH AND PHYSIOLOGICAL RESPONSES OF 6 C_4 TURFGRASSES TO SALINITY.....57

ABSTRACT.....57
INTRODUCTION.....59
MATERIALS AND METHODS.....61

RESULTS....66
DISCUSSION....96

CHAPTER VI. SALT TOLERANCE OF THE COASTAL SALT MARSH GRASS *SPOROBOLUS VIRGINICUS* (L.) KUNTH....104

ABSTRACT....104
INTRODUCTION....105
MATERIALS AND METHODS....107
RESULTS....109
Growth....109
Ion Relations....112
Osmotic Adaptation....126
Compatible Solutes....130
DISCUSSION....134

CHAPTER VII. CONCLUSIONS....143

APPENDIX A (CHAPTER III)....149

APPENDIX B (CHAPTER V)....154

APPENDIX C (CHAPTER VI)....164

APPENDIX D 171

REFERENCES 173

LIST OF TABLES

Table		Page
1	Turfgrasses evaluated for salinity tolerance	26
2	Ranking of turfgrasses according to slope of linear regression coefficients of relative shoot dry weight on salinity	30
3	Number and size of salt glands on the adaxial surface of leaves of *Zoysia matrella* and *Z. japonica* grown in nutrient solutions containing 100 *mM* NaCl	51
4	Concentration of Na^+, K^+, Ca^{2+}, and Mg^{2+} in unrinsed and rinsed leaves of *Zoysia matrella* and *Z. japonica* grown in nutrient solutions containing 200 *mM* NaCl	53
5	Turfgrasses evaluated for salinity tolerance	62
6	Shoot K^+/Na^+ ratios as influenced by NaCl concentrations of 1 and 400 *mM*	82
7	Shoot/root ratios of Na^+ and Cl^- as influenced by 1 and 400 *mM* NaCl	85
8	Pearson correlation coefficients among selected variables	95
9	The possible osmotic contributions of glycinebetaine and proline to cytoplasmic osmotic adjustment in grasses grown under 400 *mM* NaCl	102
10	Shoot K^+/Na^+ ratios, root media K^+/Na^+ ratios, and shoot selectivity ratios ($S_{K,Na}$) for K^+ at different salinities	118
11	Concentration of Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^- in rinsed and unrinsed leaves of *Sporobolus virginicus* grown under varying salinities	127
12	Shoot glycinebetaine and proline contents and their possible osmotic contributions to cytoplasmic osmotic adjustment	137

Appendix Table Page

13 Analysis of variance for regressing root dry weight on salinity across grasses.....149

14 Analysis of variance for regressing shoot Na^+ on salinity across grasses.....149

15 Analysis of variance for regressing shoot Cl^- on salinity across grasses.....150

16 Analysis of variance for regressing root Na^+ on salinity across grasses.....150

17 Analysis of variance for regressing root Cl^- on salinity across grasses.....151

18 Analysis of variance for regressing shoot K^+ on salinity across grasses.....151

19 Analysis of variance for regressing root K^+ on salinity across grasses.....152

20 Analysis of variance for regressing leaf sap osmolality on salinity across grasses.....152

21 Analysis of variance for regressing shoot tissue water content on salinity across grasses.....153

22 Analysis of variance for regressing shoot growth rate on salinity across grasses.....154

23 Analysis of variance for regressing relative shoot growth on salinity across grasses.....154

24 Analysis of variance for regressing shoot visual quality rating on salinity across grasses.....155

25 Analysis of variance for regressing shoot fresh weight/dry weight on salinity across grasses.....155

26 Analysis of variance for regressing leaf sap osmolality on salinity across grasses.....156

27 Analysis of variance for regressing shoot Na^+ on salinity across grasses.....156

28 Analysis of variance for regressing shoot Cl^- on salinity across grasses.....157

29 Analysis of variance for regressing shoot Na^+ (on a tissue water basis) on salinity across grasses.....157

Appendix Table | Page

30 Analysis of variance for regressing on shoot Cl^- (on a tissue basis) salinity across grasses.....158

31 Analysis of variance for regressing shoot K^+ on salinity across grasses.....158

32 Analysis of variance for regressing on shoot Ca^{2+} salinity across grasses.....159

33 Analysis of variance for regressing shoot Mg^{2+} on salinity across grasses.....159

34 Analysis of variance for regressing root Na^+ on salinity across grasses.....160

35 Analysis of variance for regressing root Cl^- on salinity across grasses.....160

36 Analysis of variance for regressing root K^+ on salinity across grasses.....161

37 Analysis of variance for regressing root Ca^{2+} on salinity across grasses.....161

38 Analysis of variance for regressing root Mg^{2+} on salinity across grasses.....162

39 Analysis of variance for regressing shoot proline on salinity across grasses.....162

40 Analysis of variance for regressing shoot glycinebetaine on salinity across grasses.....163

41 Analysis of variance for regressing shoot trigonelline on salinity across grasses.....163

42 Analysis of variance for regressing shoot growth rate on salinity.....164

43 Analysis of variance for regressing root dry weight on salinity.....164

44 Analysis of variance for regressing shoot Na^+ on salinity.....165

45 Analysis of variance for regressing shoot Cl^- on salinity.....165

46 Analysis of variance for regressing root Na^+ on salinity.....165

47 Analysis of variance for regressing root Cl^- on salinity.....166

Appendix Table Page

48 Analysis of variance for regressing shoot K^+ on salinity.....166

49 Analysis of variance for regressing root K^+ on salinity.....166

50 Analysis of variance for regressing shoot Ca^{2+} on salinity.....167

51 Analysis of variance for regressing shoot Mg^{2+} on salinity.....167

52 Analysis of variance for regressing root Ca^{2+} on salinity.....167

53 Analysis of variance for regressing root Mg^{2+} on salinity.....168

54 Analysis of variance for regressing shoot soluble carbohydrates on salinity.....168

55 Analysis of variance for regressing leaf sap osmolality on salinity.....168

56 Analysis of variance for regressing shoot fresh weight/dry weight on salinity.....169

57 Analysis of variance for regressing shoot proline on salinity.....169

58 Analysis of variance for regressing shoot glycinebetaine on salinity.....169

59 Analysis of variance for regressing shoot trigonelline on salinity.....170

60 Recovery trials of glycinebetaine added to samples of bermudagrass grown under low salinity.....171

LIST OF FIGURES

Figure		Page
1	Root dry weight as influenced by NaCl level	31
2	Shoot Na^+ concentration, expressed on a tissue dry weight basis, as influenced by NaCl level	33
3	Shoot Cl^- concentration, expressed on a tissue dry weight basis, as influenced by NaCl level	34
4	Root Na^+ concentration, expressed on a tissue dry weight basis, as influenced by NaCl level	35
5	Root Cl^- concentration, expressed on a tissue dry weight basis, as influenced by NaCl level	36
6	Shoot K^+ concentration, expressed on a tissue dry weight basis, as influenced by NaCl level	38
7	Root K^+ concentration, expressed on a tissue dry weight basis, as influenced by NaCl level	39
8	Leaf sap osmolality as influenced by NaCl level	40
9	Shoot tissue water content as influenced by NaCl level	42
10	Photomicrograph (32X) of a) adaxial and b) abaxial leaf surfaces of *Zoysia japonica*	48
11	Scanning electron micrographs (587X) of adaxial leaf surfaces of a)*Zoysia matrella* and b)*Z. japonica*	49
12	Scanning electron micrographs of salt glands on adaxial leaf surfaces of a)*Zoysia matrella* (1900X) and b)*Z. japonica* (2200X)	50
13	Scanning electron micrograph of abaxial leaf surface of *Zoysia japonica* (587X)	52
14	Leaf sap osmolalities of *Zoysia matrella* and *Zoysia japonica* as affected by increasing salinity of the growing media	55
15	Shoot growth rates (g dry wt. $week^{-1}$) as influenced by NaCl concentration	67
16	Relative shoot growth rates, expressed as % of control, as influenced by NaCl concentration	68

Figure Page

17 Shoot visual quality ratings as influenced by NaCl concentration....70

18 Shoot fresh weight/dry weight ratios as influenced by NaCl concentration....71

19 Leaf sap osmolalities as influenced by NaCl concentration....73

20 Shoot Na^+ concentrations, expressed on a dry weight basis, as influenced by NaCl concentration....74

21 Shoot Cl^- concentrations, expressed on a dry weight basis, as influenced by NaCl concentration....75

22 Shoot Na^+ concentrations, expressed on a tissue water basis, as influenced by NaCl concentration....76

23 Shoot Cl^- concentrations, expressed on a tissue water basis, as influenced by NaCl concentration....77

24 Shoot K^+ concentrations, expressed on a dry weight basis, as influenced by NaCl concentration....78

25 Shoot Ca^{2+} concentrations, expressed on a dry weight basis, as influenced by NaCl concentration....79

26 Shoot Mg^{2+} concentrations, expressed on a dry weight basis, as influenced by NaCl concentration....80

27 Root Na^+ concentrations, expressed on a dry weight basis, as influenced by NaCl concentration....83

28 Root Cl^- concentrations, expressed on a dry weight basis, as influenced by NaCl concentration....84

29 Root K^+ concentrations, expressed on a dry weight basis, as influenced by NaCl concentration....86

30 Root Ca^{2+} concentrations, expressed on a dry weight basis, as influenced by NaCl concentration....87

31 Root Mg^{2+} concentrations, expressed on a dry weight basis, as influenced by NaCl concentration....88

32 Ion concentrations in leaves of grasses grown in 200 *mM* NaCl....90

33 Shoot proline concentrations, expressed on a dry weight basis, as influenced by NaCl concentration....91

Figure Page

34 Shoot glycinebetaine concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.....92

35 Shoot trigonelline concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.....94

36 Shoot growth rate, expressed as g dry wt. $week^{-1}$, as influenced by NaCl level.....110

37 Root dry weight as influenced by NaCl level.....111

38 Shoot Na^+, expressed on a dry weight basis, as influenced by NaCl level.....113

39 Shoot Cl^-, expressed on a dry weight basis, as influenced by NaCl level.....114

40 Root Na^+, expressed on a dry weight basis, as influenced by NaCl level.....115

41 Root Cl^-, expressed on a dry weight basis, as influenced by NaCl level.....116

42 Shoot K^+, expressed on a dry weight basis, as influenced by NaCl level.....117

43 Root K^+, expressed on a dry weight basis, as influenced by NaCl level.....120

44 Shoot Ca^{2+}, expressed on a dry weight basis, as influenced by NaCl level.....121

45 Shoot Mg^{2+}, expressed on a dry weight basis, as influenced by NaCl level.....122

46 Root Ca^{2+}, expressed on a dry weight basis, as influenced byNaCl level.....123

47 Root Mg^{2+}, expressed on a dry weight basis, as influenced by NaCl level.....124

48 Shoot soluble carbohydrates, expressed on a dry weight basis, as influenced by NaCl level.....125

49 Leaf sap osmolality as influenced by NaCl level.....128

50 Shoot fresh weight/dry weight as influenced by NaCl level.....129

Figure Page

51 Shoot solute concentrations, expressed on a tissue water basis, as influenced by NaCl level.....131

52 Calculated contributions of solutes to the osmotic adjustment of shoots at different salinities, assuming an osmotic coefficient of 0.9 for inorganic ions and 1.0 for sugars.....132

53 Shoot proline, expressed on a dry weight basis, as influenced by NaCl level.....133

54 Shoot glycinebetaine, expressed on a dry weight basis, as influenced by NaCl level.....135

55 Shoot trigonelline, expressed on a dry weight basis, as influenced by NaCl level.....136

56 Representative HPLC chromatograms for glycinebetaine.....172

CHAPTER I

INTRODUCTION

There is an increasing need for salt tolerant turfgrasses in Hawaii, as well as in other states. Coastal areas suffer from the effects of salt spray and occasional salt water inundations. In addition, increased demand on limited water resources has resulted in salt water intrusion into fresh water wells, some of which are used for turfgrass irrigation. In semi-arid areas, such as the southwestern United States, salt accumulation in soils is a major problem. In addition, water sources utilized for irrigation are frequently brackish, exacerbating the problem.

An understanding of the physiology of salt tolerance of turfgrasses is important for an effective approach to the salinity problem, which will include improved plant culture and irrigation management, as well as the selection of improved genotypes. Unfortunately, efforts in breeding for salt tolerance have been hindered, due to the incomplete knowledge of the effects of salinity on plants, the vague, nonspecific effects, other than growth reduction, of moderate salt stress, and the interactions of environment with salt stress (Shannon, 1984). The identification of specific salt tolerance mechanisms would provide potential "biological markers" useful in plant selection. Such markers include characters that are associated with salt resistance and can be used for screening salt resistant plants in breeding populations. The lack of such markers in most crop plants is

one of the biggest problems of the conventional plant breeder at the present time (Shannon, 1980).

Salinity tolerance is complicated, involving a number of independent physiological characteristics. Knowledge of these characteristics would allow breeders to utilize a "building block" approach, ie. the combination of several characteristics to achieve a resistant variety (Yeo and Flowers, 1984).

Though there have been a number of reports concerning basic responses of turfgrasses to salinity, there is little information available concerning physiological responses to salinity. Such information would aid in the understanding of salinity tolerance mechanisms or strategies operating in these grasses. A salt tolerant coastal grass, *Sporobolus virginicus* (L.) Kunth was included in these studies. *S. virginicus* is closely related to the bermudagrasses and zoysiagrasses, therefore an understanding of the adaptations to salinity of *S. virginicus* might shed light on the salt tolerance strategies utilized by other grasses in these studies.

CHAPTER II

LITERATURE REVIEW

There is an extremely wide range of salt tolerance in higher plants, from sensitive glycophytes which tolerate almost no salinity to extreme halophytes that thrive in full strength sea water. Although halophytic plants are from a variety of families, many are members of three families, namely the Chenopodiaceae, Poaceae, and Compositae (Jefferies and Rudmik, 1984).

Salt tolerance is often measured as a relative reduction in yield upon exposure to increasing soil salinity (Shannon, 1984). Maas and Hoffman (1977) fit linear regression equations to yield data of crops grown under increasing salinities. Slope values were equal to the relative yield reduction per unit salinity increase, a measure of relative salt tolerance. Dudeck *et al.*, (1983) also used linear slope coefficients, as well as the predicted EC value (solution salinity) resulting in 50% growth reduction, to compare relative salt tolerance of bermudagrass (*Cynodon* Spp. (L.) Pers. Burtt-Davey) cultivars.

Salinity Tolerance in Turfgrasses

Within the Poaceae, there are large differences in salinity tolerance between genera, species, and even cultivars. Maas and Hoffman (1977) reported a 50% growth reduction for lovegrass (*Eragrostis* spp. Beauv.) at 7.9 dS m^{-1} (decisiemens per meter) external solution conductivity, and for common bermudagrass (*Cynodon dactylon* (L.) Pers.) at 14.7 dS m^{-1}. Shoot growth was reduced 50% in *Spartina foliosa* Trin.,

a coastal salt marsh grass, when grown in greater than 50% seawater (approximately 25 dS m^{-1}) (Phleger, 1971).

Lunt *et al.*, (1961) found differences in salinity tolerance among C_3 (cool season) turfgrasses. 'Seaside' creeping bentgrass (*Agrostis palustris* Huds.) and 'Alta' tall fescue (*Festuca arundinaceae* Schreb.) were relatively tolerant to salinity, while Kentucky bluegrass (*Poa pratensis* L.) and 'Highland' colonial bentgrass (*Agrostis tenuis* Sibth.) were much more sensitive. Younger *et al.*, (1967) reported that survival under saline conditions was highly correlated to speed of recovery after removal of salt stress in 7 creeping bentgrass cultivars.

Among C_4 (warm season) turfgrasses, the bermudagrasses have been considered the most tolerant (Beard, 1973) and are widely used in areas where salinity is often a problem (Ackerson and Youngner, 1975), although the zoysiagrasses (*Zoysia* Spp. (L.) Merr. Steud.) and St. Augustinegrass (*Stenotaphrum secundatum* Walt.) are also considered relatively salt tolerant. Younger and Lunt (1967) tested 9 bermudagrass cultivars, using salinities of up to 340 meq L^{-1} NaCl (32 dS m^{-1}) (U.S.D.A., 1969). 'Sunturf' and 'Tifway' were more tolerant than 'Tifgreen' and common bermudagrass, with 50% growth reductions occuring at 34, 33, 29, and 25 dS m^{-1}, respectively (calculated from reported data). Dudeck *et al.*, (1983) found differences in 8 bermudagrass cultivars at salinities of up to 32.5 dS m^{-1}. 'Tifdwarf' and 'Tifgreen' were more tolerant in this case, with 50% growth reductions occuring at about 22 dS m^{-1}. 'Tifway' and common bermudagrass were less tolerant, with 50% growth reductions occuring at 18 dS m^{-1}. Although top growth

was severely reduced at the highest salt level of 32 dS m^{-1}, no mortality was noted in any cultivar.

Recently, attention has been drawn to seashore paspalum (*Paspalum vaginatum* Swartz.), a C_4 turfgrass which seems to be more salt tolerant than bermudagrass. In Australia the grass has been used for bogs and seepage areas which stay wet with salty water (Malcolm and Laing, 1969). In Florida, the recently introduced cultivar 'Adalayd' was reported to equal the quality of the hybrid bermudagrasses when used on a golf course, performing well at cutting heights as low as 5/32 inch (Guiot and Flynn, 1983). On a golf course fairway in San Clemente, California, 'Futurf', another recently introduced seashore paspalum cultivar, was found growing on soils with ECe readings ranging from 40 to 45 dS m^{-1} (Henry *et al.*, 1979). Dudeck and Peacock (1985) reported a selection of seashore paspalum having a 50% growth reduction at 28.6 dS m^{-1}, which was higher than the 22 dS m^{-1} previously reported by the authors for 'Tifdwarf' and 'Tifgreen' bermudagrass. However, this comparison may not be valid, as they were separate experiments, and environmental conditions probably varied.

Morphological Adaptations to Salinity

Changes in growth characteristics (morphology) are common in plants subjected to salt stress. Such plants are usually stunted and may have darker green or blue-green leaves, which, in some cases, are thicker and more succulent (Maas and Hoffman, 1977). Also, leaf size is often smaller, which may be the result of water deficit caused by the salt stress (Hsiao and Bradford, 1983). Water stressed perennial

ryegrass (*Loleum perenne* L.) leaves were smaller, with deeper ridging on their adaxial surfaces (Jones *et al.*, 1980). Transpiration rates decreased with increasing salinity in two perennial wheatgrasses, partly due to an increase in the epicuticular wax covering (Gorham *et al.*, 1985b). Smaller leaves with thicker cuticles result in lower transpiration rates, which enable the plant to better maintain cell turgor (Jefferies and Rudmik, 1984), and also to limit the amount of salt transported to the shoot (Levitt, 1980).

An increase in the root/shoot ratio is also a common morphological response to salinity stress (Maas and Hoffman, 1977). Younger and Lunt (1967) found that, though top growth decreased with increasing salinity, root growth increased under intermediate salt treatments in all 9 bermudagrass cultivars studied. Dudeck *et al.*, (1983) and Dudeck and Peacock (1985) also found that root growth was stimulated, and top growth suppressed, under intermediate salinities in 7 of 8 bermudagrass cultivars, and 1 of 4 seashore paspalum selections studied. An increase in the root/shoot ratio may be an adaptation to salinity, increasing the capacity for water absorption in relation to transpiration, thereby increasing moisture availability to the plant under osmotic stress (Bernstein and Hayward, 1958; Younger and Lunt, 1967).

Osmotic Relations

Plants face two major obstacles to growth under saline conditions: a) water stress arising from the more negative water potential (due to the lowered osmotic potential) of the rooting medium, b) specific ion toxicity (or imbalance) usually associated with excessive Na^+ and Cl^-

uptake (Gorham *et al.*, 1985b). These obstacles are related in the sense that the solutions to each are mutually exclusive. In a saline environment a plant needs to adjust osmotically; it is therefore liable to suffer from ion excess if it accumulates the ions necessary for adjustment and from water deficit if it does not (Yeo, 1983).

There is a direct relationship between salt stress and water stress in plants. Since cell membranes are freely permeable to water, it is not possible for cells to avoid the osmotic stress caused by increased salinity of the soil solution. As the osmotic potential of the soil solution becomes more negative, the cell's first response is a loss of turgor, and a resultant growth reduction, as growth is intimately tied to maintenance of cell turgor (Bernstein, 1961; Neumann *et al.*, 1988). The maintenance of cell turgor requires a sufficient increase in sap osmolality to compensate for the external osmotic stress, a process called osmoregulation, or osmotic adjustment (Levitt, 1980; Hellebust, 1976). Osmolality= Ø x n x molality, where Ø=osmotic coefficient, a measure of the deviation of the solution from ideality, and n=number of particles into which a solute molecule dissociates (Wyn Jones and Gorham, 1983). The solutes accumulated for osmotic adjustment in the shoots can be either inorganic ions or soluble organic compounds synthesized by the plant, or more generally a combination of both.

Exact causes of shoot growth reduction in salt-stressed plants, and particularly in monocots, remain elusive (Munns *et al.*, 1982; Greenway and Munns, 1983; Yeo, 1983). A number of proposals have been put forth:

a) Inadequate respiratory system to provide sufficient energy for active transport of ions across membranes, or alternatively, to an insufficient number of carriers, needed for the fast rate of ion uptake required for cell elongation under saline conditions (Yeo, 1983; Greenway and Munns, 1983).

b) Reduced photosynthetic capacity, including increased stomatal and mesophyll resistance, due either to adverse osmotic relations or ion toxicity (Robertson and Wainwright, 1987).

c) A buildup of salts in cell walls (apoplast) which would effectively reduce cell turgor by water diffusion out of cells (Oertli, 1968; Flowers and Yeo, 1986).

d) A buildup of salts in the cytoplasm to toxic levels at high salinity if vacuolar ion compartmentation becomes inadequate (Storey and Wyn Jones, 1979).

Ion Uptake/Exclusion

As was mentioned, plants are sensitive to salinity because of either excess Na^+ or Cl^- in the cytoplasm, with resulting ion toxicities or imbalances, or to water deficits and related effects caused by osmotic stress. "Salt excluding" plants, including most monocotyledonous plants, tend to exclude salt from their shoots, thereby minimizing toxic effects (Kramer, 1984). Monocots, including the Poaceae, tend to restrict the entry of inorganic ions to the shoots, but as a consequence may suffer from reduced growth rates under saline conditions, due to cell dehydration (Albert and Popp, 1977; Gorham *et al.*, 1980; Gorham *et al.*, 1985b). However, shoot K^+ concentrations are

generally kept high relative to Na^+. Gorham *et al.*, (1980) found that the K^+/Na^+ ratio exceeded 1 in halophytic grasses collected from salt marshes. A high affinity for K^+ in shoots may be interpreted as a requirement for a minimum cytoplasmic K^+, possibly associated with the K^+ requirement of protein synthesis (Wyn Jones *et al.*, 1979).

Salt exclusion in many grasses may be achieved by a very efficient selectivity for K^+ over Na^+ during root absorption. There is evidence for selective K^+ absorption-Na^+ exclusion/compartmentation by the root cortical cells or endodermis (Kramer, 1984; Jeschke, 1984). The detailed transport mechanisms for Na^+ and K^+ across the plasmalemma and tonoplast are not known, but the net effect is to selectively take up K^+ into the cytoplasm, while extruding Na^+ both into the vacuole and the external medium. The model for selective K^+-Na^+ exchange includes H^+-ATPases in both membranes, which generate an electrical potential difference and a protonmotive force across the membranes. The electrical charge difference is compensated for by an influx of K^+ into the cytoplasm at specific K^+ uniports, while the proton gradient provides energy for extrusion of Na^+ across both plasmalemma and tonoplast by H^+-Na^+ antiports (Garbario and DuPont, 1988; Martinoia *et al.*, 1986; Jeschke, 1984; Hurkman *et al.*, 1988). X-ray microanalysis of the halophytic grass *Puccinellia peisonis* showed a decreasing gradient of Na^+ and an increasing gradient of K^+ in the roots, from the outer cortex through the endodermis to the stele, which led to a high selectivity for K^+ over Na^+ in transport to the xylem (Levitt, 1980). Barley (*Hordeum vulgare* L.) actively extrudes Na^+ from roots in exchange for K^+, resulting in preferred K^+ transport to the shoot (Jeschke and

Stelter, 1973; Jeschke, 1984). Salinity also induced a Na^+/K^+ exchange in the tonoplast membrane of barley roots (Garbarino and DuPont, 1988).

Salt tolerance in closely related species is often associated with Na^+ exclusion from shoots, particularly in glycophytic grasses (Weimberg, 1986). Greenway (1962) found in barley that the least resistant cultivar had higher Na^+ and Cl^-, but lower K^+ shoot concentrations than two resistant cultivars. Storey and Wyn Jones (1978b) found a salt resistant barley cultivar to have a higher affinity for K^+, coupled with a better ability to regulate Na^+ and Cl^- contents of shoots under salt stress. Although less Cl^- was found in the roots of the sensitive cultivar compared to the resistant one, more was found in the shoots, indicating that Cl^- was more readily transported to the shoots. Greater salt tolerance in three barley cultivars was related to higher shoot K^+/Na^+ ratios and higher K^+ selectivity ratios ($S_{K,Na}$=[K^+ in plant][Na^+ in medium]/[Na^+ in plant][K^+ in medium]) (Pitman, 1969). Shannon (1980), in screening 32 lines of tall wheatgrass (*Agropyron elongatum* Host Beauv.) for salt tolerance found that tolerance was associated with both restricted accumulation of Na^+, Cl^-, and Ca^{2+} in the shoots, and the maintenance of high shoot K^+/Na^+ ratios (greater than 1). Similar results were found for clones of bentgrass (*Agrostis stolonifera* Beauv.) and red fescue (*Festuca rubra* L.) (Hannon and Barber, 1972). Salt tolerant populations of *A. stolonifera* collected from maritime habitats had lower shoot Na^+ concentrations and higher K^+/Na^+ ratios than inland populations when both were grown over a range of salinities. Plants may succeed in excluding salt only from the shoots. In *Eragrostis tenella* Staph. a large part of the Na^+ absorbed

by the roots is retained there, presumably accumulating in the cell vacuoles (Levitt, 1980).

Alternatively, Na^+ may be taken up by the roots, followed by reabsorption from the xylem via mature xylem parenchyma cells in the roots or shoots, and possible translocation back to the soil, as in maize (*Zea mays* L.) (Shone *et al.*, 1969; Jeschke, 1979). Apparently this strategy of K^+/Na^+ selectivity is utilized only by extremely salt-sensitive Na^+ excluders, such as maize and rice (Yeo *et al.*, 1977).

In some highly adapted halophytes, salt exclusion is achieved by an extrusion mechanism located in leaf salt glands or bladders. Several *Atriplex* species possess epidermal bladders into which excess salt is secreted. *Atriplex halimus* also has vesiculate leaf hairs which remove salt from the remainder of the leaf, preventing toxicities in the parenchyma and vascular cells (Levitt, 1980; Kramer, 1984). In this way a nearly constant salt content is maintained in the leaf cells. *Diplache fusca* Beau., known as Kaller grass in Pakistan, selectively secretes Na^+ and Cl^- from leaf salt glands, resulting in shoot selectivity for K^+ over Na^+ (Sandhu *et al.*, 1981). Salt glands which selectively secrete NaCl have also been reported in the European cordgrass, *Spartina townsendii* H. and J. Groves, allowing a high K^+/Na^+ ratio to be maintained in the shoots of this halophytic grass, even though there is a large, nonselective NaCl uptake by the roots (Wyn Jones and Storey, 1978b).

Within the Poaceae, salt glands have been reported to occur in over 30 species of the tribes Chlorideae, Eragrosteae, Aeluropodeae, and Pappophoreae (Leonard, 1983; Lipshchitz and Waisel, 1974; Taleisnik and

Anton, 1988), all members of the subfamily Chloridoideae (Gould and Shaw, 1983). These salt glands are invariably modified epidermal trichomes consisting of a basal, and cap cell, being structurally distinct from those of dicotyledonous halophytes (Fahn, 1988).

Ion exclusion protects grasses from the toxic effects of salinity, but also contributes to osmotic imbalances, resulting in partial tissue dehydration and very slow growth under high salinity (Greenway and Munns, 1980). Osmotic adaptation in grasses is aided by the accumulation of sugars, with accumulations of up to 200 mmol L^{-1} of tissue water in some halophytic grasses (Albert and Popp, 1977). In plant surveys, monocotyledonous plants, and grasses in particular, tended to accumulate free sugars in response to salt stress (Gorham *et al.*, 1980; Gorham *et al.*, 1981). The large amounts of sugars found suggested that a substantial portion must be located in the vacuoles, as well as in the cytoplasm. Gorham *et al.* (1981), in surveying a number of monocotyledonous plants, found levels of soluble carbohydrates high enough to partially replace the turgor-generating potential of the inorganic ions, which, to some extent, were excluded from these plants. Osmotic adjustment was aided by the accumulation of sucrose in *Elytrigia juncea* L. and *Leymus sabulosis* Bieb., two perennial grasses of the tribe Triticeae (Gorham *et al.*, 1984; Gorham *et al.*, 1985a). Shannon (1980) reported that the soluble sugar content was substantially higher in the leaf tissues of salt tolerant tall wheatgrass lines than in sensitive ones grown under saline conditions.

The disadvantage of osmotic adjustment on a whole-cell basis by means of organic compounds is its high energy cost which may result in

growth reduction (Läuchli, 1984). The use of sugars as osmotica is inefficient, in terms of both energy and mass (or carbon demand) compared to inorganic ions. The accumulation of one osmol (osmotically active mole) hexose has been calculated to require 54 mol ATP, compared to 0.54 mol ATP for the transport of one osmol of NaCl to the shoot (Gorham *et al.*, 1980). Furthermore, the presence of sufficient hexose to generate an osmotic potential of 300 mOsmol kg^{-1} (a moderate osmotic contribution) of C_6 sugars in the cell sap would take up 20-30% of the dry weight of the tissue (Wyn Jones, 1981).

For turfgrasses, information on ion status under increasing salinities is very limited. Lunt *et al.*, (1961) reported that Na^+ and Cl^- seemed to increase approximately linearly with increasing salt concentrations of the irrigation water in 4 cool season turfgrasses. The rate of accumulation of Cl^- in 'Highland' colonial bentgrass and Kentucky bluegrass seemed to be much more rapid than for the more tolerant 'Alta' tall fescue and 'Seaside' creeping bentgrass , though tissue ion data for Kentucky bluegrass and 'Highland' bentgrass was not taken for the higher salinity treatments. Total shoot and root ion concentrations changed little with increasing salinity in the bermudagrass cultivar 'Santa Ana', indicating restricted root ion uptake (Ackerson and Younger, 1975). In addition, total nonstructural carbohydrates increased in crown tissue with increasing salinity. The authors suggested that organic osmotica (sugars) may be substituting for inorganic ions, preventing toxicity. However, the analysis method used included starch, which is not osmotically active. Dudeck *et al.*, (1983) grew eight bermudagrass cultivars under salinities of up to 32.5 dS m^{-1}

NaCl (approximately 319 *mM* NaCl). Shoot sodium increased, while K^+ decreased with increasing NaCl in shoots of all cultivars, but the total (Na^++K^+) concentration remained constant, indicating ion exclusion. Although cultivars differed in their total Na^++K^+ in shoots, there appeared to be no relationship between ion content and salt tolerance. In a study including 4 seashore paspalum selections, it was invariably found that Na^+ and Cl^- shoot concentrations increased, while K^+ concentrations decreased with increasing salinity (Dudeck and Peacock, 1985). The most tolerant selection reached maximum shoot Na^+ and Cl^- concentrations at 31 dS m^{-1} external salinity (approximately 304 *mM* NaCl), while Na^+ and Cl^- concentrations in the other selections continued a linear increase beyond 31 dS m^{-1}. The authors attributed salt tolerance in the most tolerant selection to an ability to exclude further uptake of Na^+ and Cl^- after maximum levels were reached, while other cultivars were unable to do so.

Though the majority of salt tolerant grasses have been classified as "salt excluders", this is misleading (Greenway and Munns, 1980). *Leymus sabulosis* L. and *Elytrigia juncea* L., two perennial grasses of the tribe Triticeae, survived and continued to grow in 250 *mM* NaCl. Their tolerance was associated with an ability to tightly control osmotic adjustment by strictly regulating the influx of NaCl. As a result, the change in sap osmotic potential closely followed the change in external osmotic potential (Gorham *et al.*, 1985a; Gorham *et al.*, 1984). Similar results were found for 32 lines of tall wheatgrass (Shannon, 1980), red fescue (Hannon and Barber, 1972), and Kallar grass (Sandhu *et al.*, 1981).

Members of the Poaceae show wide variability in their responses to salinity, some relying more on ions for osmotic adjustment than others. In *Spartina townsendii* H. and J. Groves, a halophytic grass, there is a net accumulation of Na^+ and Cl^- in shoots with increasing salinity, leading to a steady increase in tissue osmotic potential. However, at very high salinities (over 500 *mM* NaCl) tissue osmotic adjustment was attributed to a decrease in the tissue water content (tissue dehydration) (Storey and Wyn Jones, 1978a). In contrast, barley was less able to utilize ions for osmotic adjustment at increasing salinities. Though Na^+ and Cl^- did accumulate, the K^+ content fell almost in proportion, so there was only a small net ion increase in the shoots. In this case, tissue dehydration occured at much lower salinities (less than 250 *mM* NaCl), resulting in growth inhibition, and osmotic adjustment was due largely to tissue dehydration (Storey and Wyn Jones, 1978b). In comparison with *Spartina townsendii*, barley appears to have less ability to use Na^+ and Cl^- for osmotic adjustment, perhaps due to less efficient ion compartmentation in vacuoles (Wyn Jones and Storey, 1978a,b). This interpretation implies that efficient ion compartmentation is a crucial factor in the salt tolerance of plants (Greenway and Munns, 1980; Wyn Jones, 1981).

Intracellular Solute Compartmentation

In contrast to salt excluding plants, certain dicotyledonous halophytes, such as members of the Chenopodiaceae and Caryophyllaceae accumulate salt to very high levels under salinity, resulting in increased shoot succulence and more rapid growth at intermediate

salinities (Flowers, 1985; Gorham *et al.*, 1985b). Even so, their Na^+ and Cl^- uptake is under tight control (Wyn Jones, 1981; Munns *et al.*, 1983). However, all salt tolerant plants, including the grasses, utilize inorganic ions for a large part of their osmotic adjustment, as the ability to accumulate organic solutes on a whole cell basis is limited (Kramer, 1984; Wyn Jones and Storey, 1978a; Levitt, 1980).

The enzyme systems of plants in general are similar in sensitivity to high Na^+ and Cl^- concentrations, being inhibited at concentrations above 100-200 *mM* (Field, 1976; Flowers *et al.*, 1977; Wyn Jones *et al.*, 1979). Consequently, tolerant plants under high salt conditions, while accumulating ions for osmotic adjustment, must restrict the level of ions in the cytoplasm. The widely accepted hypothesis is that Na^+ and Cl^- are actively pumped through the tonoplast into the cell vacuole, where they are sequestered, a process called "ion compartmentation" (Jeschke, 1984; Greenway and Munns, 1980). In leaves of the Australian salt bush (*Atriplex spongiosa*) the cytoplasmic concentration of Na^+ and Cl^- was estimated to be in the range of 75-150 *mM* compared to a concentration in the vacuole of 700 *mM*, when grown in a solution containing 600 *mM* NaCl (Pittman, 1984). There is evidence in cereals and other plants for a vacuole/cytoplasm exchange of K^+ for Na^+ during compartmentation to maintain a relativaly constant cytoplasmic K^+ concentration of about 100 *mM*, which is required for the functioning of protein synthesizing enzymes (Jeschke, 1979; Wyn Jones *et al.*, 1979). Kinetic analysis of K^+ and Na^+ efflux from barley roots showed that the ratio of Na^+/K^+ in the cytoplasm was 0.09, while in the vacuole it was 0.3 (Pittman, 1984). Direct sub-cellular analysis using transmission

electron microscopy was used on leaf segments of *Suaeda maritima* grown under 340 *mM* NaCl. Preparation by freeze-substitution in acetone and embedment in resin containing known concentrations of Na^+, K^+, and Cl^- made sub-cellular localization of ions possible. Vacuoles accumulated the large majority of Na^+ and Cl^-, both in terms of total amounts and concentrations (Harvey *et al.*, 1981). Similarly, electron-probe X-ray microanalysis of bulk-frozen and fractured preparations showed much higher K^+/Na^+ ratios in the cytoplasm than in the vacuoles of young leaf cells (Gorham and Wyn Jones, 1983). X-ray microanalysis of frozen-hydrated tobacco cells adapted to salt indicated that Na^+ and Cl^- were compartmentalized in the vacuole, at concentrations of 780 and 624 *mM*, respectively, while cytoplasmic concentrations were maintained at 96 *mM* (Binzel *et al.*, 1988).

The mechanisms of ion transport across the tonoplast are not known with certainty. Sodium transport across the membranes appears to be mediated by Na^+/H^+ antiport, and the ultimate motor of K^+/Na^+ exchange is an ATP-dependent proton extrusion pump (Jeschke, 1979; Jeschke, 1984). This pump generates an electrical potential which is dissipated in part by an influx of K^+ at a specific site. Extrusion of Na^+ against the electrochemical gradient is suggested to occur in exchange for protons (H^+-Na^+ antiport) which are then reextruded by the proton pump (Jeschke, 1984). For the tonoplast, Na^+ and/or Cl^- are actively transported into the vacuole, and occluded there irreversibly (Greenway and Munns, 1983). There is evidence for the existence of tonoplast ATPases in a number of species (Lüttge and Smith, 1984). Evidence has been found for a Na^+/H^+ antiport in barley tonoplast vesicles. The

activity of the antiport was observed only in membranes from roots that were grown in NaCl (Garbarino and DuPont, 1988).

Compatible Solutes

All cellular compartments are subjected to equal osmotic potential and turgor. As ions accumulate in the vacuole, lowering its osmotic potential, the cytoplasm must equilibrate. This has led to the concept of "compatible solutes" or "cytosolutes" (Wyn Jones, 1981), certain organic compounds accumulated in the cytoplasm which serve specifically to balance osmotic potential without inhibiting enzyme function (Levitt, 1980; Gorham *et al.*, 1985).

A number of possible compatible cytosolutes have been proposed in higher plants (Wyn Jones, 1981). However, in some cases the evidence is circumstantial, and has yet to be produced in each case to show that accumulation is of adaptive value and not a reflection of impaired metabolism. The cytoplasmic compound proposed for osmoregulation in most cases is either a nitrogen dipole, frequently an amino or imino acid derivitive, such as glycinebetaine or proline, or less frequently a small polyhydric alcohol or derivitive (Wyn Jones *et al.*, 1977; Wyn Jones, 1984).

i) Glycinebetaine

The accumulation of various compatible solutes is strongly related to taxonomic groups (Wyn Jones and Gorham, 1983; Gorham *et al.*, 1985). Two cytosolutes, proline and glycinebetaine, are often found to accumulate in salt tolerant plants of the Poaceae (Storey *et al.*, 1977;

Briens and Larher, 1982; Gorham *et al.*, 1985b). Increases in glycinebetaine of 200% were found with increases in salinity which only slightly inhibited growth in cordgrass (*Spartina townsendii*). Though other quaternary ammonium compounds were found in barley, only glycinebetaine increased in response to NaCl stress (Wyn Jones and Storey, 1978a). Glycinebetaine contents were correlated with increases of sap osmotic pressures in barley and in *Spartina townsendii* (Storey and Wyn Jones, 1977). Storey and Wyn Jones (1977), and Storey *et al.*, (1977) proposed that glycinebetaine acts as a cytoplasmic osmoticum in plant cells operating at osmotic pressures above about 350 mOsmol kg^{-1}.

ii) Proline

Proline has been suggested as being a compatible solute in many halophytes, and in particular the grasses (Stewart and Lee, 1974; Flowers *et al.*, 1977; Hasegawa *et al.*,1986). Proline accumulated in salt stressed barley and Rhodes grass (*Chloris gayana* Kunth.), but not in the dicotyledenous halophytes surveyed (Storey and Wyn Jones, 1975). However, accumulation is a common response to water stress, and may be a measure of internal water stress (Wyn Jones, 1981). Proline accumulated markedly in barley cultivars and *Spartina townsendii* following salt stress, but the final levels did not correlate with differences in salt tolerance . Both proline and glycinebetaine accumulation were highly correlated with increases in sap osmotic pressures, although proline contributed significantly only in shoots exposed to inhibitory salinities (Wyn Jones and Storey, 1978a,b). *Puccinellia maritima* (Huds.) Parls., a salt marsh grass, was reported to accumulate high

levels of proline in response to salt stress (Gorham *et al.*, 1980; Briens and Larher, 1982). Briens and Larher (1982) reported that red fescue (*Festuca rubra*) L. and *Puccinellia maritima* accumulated both sugars and proline under salt stress.

iii) Evidence of Location in Cytoplasm

Meristems and pollen contain large proportions of cytoplasm, thus are tissues in which the predictions of the model of intracellular compartmentation can be tested. However, vacuolation occurs very rapidly, and the region in which cytoplasm occupies the major volume of the cell may be no more than a few hundred μm, requiring semi-micro techniques. Both meristematic and pollen tissues contain much higher concentrations of glycinebetaine than do fully vacuolated tissues (Gorham and Wyn Jones, 1983; Gorham *et al.*, 1985b). Vacuoles isolated from beet root tissue were analysed for glycinebetaine to determine indirectly the cytoplasmic concentration. In all cases the concentration of glycinebetaine was higher in the cytoplasm (Leigh *et al.*, 1981).

iv) Evidence-Enzyme Protection

Recent evidence clearly shows that not only are glycinebetaine and proline "compatible" solutes, in that they are non-toxic, but that they may actually protect enzymes from deactivation by salts (Wyn Jones, 1984; Grumet and Hanson, 1986). Glycinebetaine partially protected malic dehydrogenase against salt inhibition (Aspinall, 1986). At 0.5 *M*, glycinebetaine and to a lesser extent proline, alleviated the inhibitory

effects of 200 *mM* NaCl on malic enzyme isolated from barley (Pollard and Wyn Jones, 1979). Glycinebetaine and proline also interact with membranes. The integrity of the membranes of red beet discs subjected to temperature stress and oxalate destabilization was improved by glycinebetaine (Jolivet *et al.*, 1983). Other studies indicate that glycinebetaine modifies fluxes of Na^+ and Cl^- across both the plasmalemma and tonoplast (Wyn Jones, 1981).

There is evidence that glycinebetaine and proline increase the affinity of certain enzymes for their substrates while exposed to inhibitory salinity levels. Incharoensakdi *et al.* (1986) found that glycinebetaine lowered the K_m of RuBp carboxylase for RuBP, which had been increased by 0.25 *M* salt in vitro. The K_m values for potassium of pyruvate kinase from a number of halophytes were reduced by 60 to 70% by the addition of glycinebetaine (Matoh *et al.*, 1988). The effects of glycinebetaine and proline on the activity of phosphoenolpyruvate carboxylase extracted from four C_4 plants (*Cynodon Dactylon, Sporobolus pungens* L., *Salsola soda* L., and *Salsola kali* L.) was tested (Manetas *et al.*, 1986). PEPCase is very sensitive to salt in vitro, being severely inhibited under NaCl concentrations of 50-100 *mM*. Both glycinebetaine and proline stabilized PEPCase from *Cynodon* and *Sporobolus* spp. against loss of activity from NaCl during the assay. Glycinebetaine was most effective, offering complete protection at 800 *mM*. PEP concentrations were kept low (.24 *mM*) to mimic estimated cytoplasmic concentrations. However, proline accelerated PEPCase inactivation due to salinity in the *Salsola* spp., whereas glycinebetaine offered protection. The levels of free proline were high in the grasses, whereas in the Chenopodiaceae

(*Salsola* spp.) they were low. Glycinebetaine levels were high in all plants. It was inferred that coevolution of PEPCase occured along with the cytosolute of choice for these species.

CHAPTER III

GROWTH RESPONSES, ION RELATIONS, AND OSMOTIC ADAPTATIONS OF ELEVEN C_4 TURFGRASSES TO SALINITY

ABSTRACT

Shortages of fresh water, coupled with soil salinization in many areas have resulted in an increased need for salt tolerant turfgrasses. This study was conducted to compare growth and physiological responses of eleven C_4 turfgrasses to salinity. Grasses were grown in solution culture in a glasshouse, with sodium chloride added to achieve salinities of 0.7, 10, 20, and 30 dS m^{-1} (0, 99, 198, and 298 *mM* NaCl). Grasses were ranked for salinity tolerance according to their relative top growth reductions with increasing salinity. Tolerant grasses included a Hawaii selection of seashore paspalum (*Paspalum vaginatum* Swartz), two St. Augustinegrasses (*Stenotaphrum secondatum* Walt.), and manilagrass (*Zoysia matrella* L.). Bermudagrasses (*Cynodon* spp. (L.) Pers. Burtt-Davey) tested were generally less tolerant to salinity. Shoot and root sodium and chloride concentrations reached very high levels in St. Augustinegrasses, and were relatively high in seashore paspalum and Japanese lawngrass (*Zoysia japonica* Steud.). In contrast, manilagrass and bermudagrasses maintained low levels of Na^+ and Cl^- under high salinity which is indicative of ion regulation, due in part to efficient leaf salt glands. Seashore paspalum maintained higher shoot and root K^+ concentrations under high salinity than did other grasses. All grasses adjusted osmotically under increasing salinity.

Although St. Augustinegrasses and seashore paspalum accumulated Na^+ and Cl^- in the shoots to relatively high levels, they maintained much higher tissue water levels than did other grasses, resulting in intermediate sap osmolalities.

INTRODUCTION

Water shortages in Hawaii, coupled with salt water intrusion into fresh water wells have resulted in a need for salt tolerant turfgrasses. In addition, coastal areas suffer from the effects of salt water spray and occasional salt water inundations.

Effects of salinity on plant growth and physiology have been reviewed (Greenway and Munns, 1980; Yeo, 1983; Gorham *et al.*, 1985b). Hazards associated with saline conditions are considered to be water stress arising from osmotic imbalances between plant and soil, and ion toxicity/imbalance associated with excessive salt uptake (Gorham *et al.*, 1985b).

Relatively little is known about the responses of C_4 (warm season) turfgrasses to salinity . Differences in relative top growth reductions under salt stress have been found among bermudagrass (Youngner and Lunt, 1967; Ramakrishnan and Nagpal, 1973; Dudeck *et al.*, 1983), and seashore paspalum cultivars (Dudeck and Peacock, 1985). An increase in root/shoot ratios under salt stress have been reported to occur in bermudagrass (Youngner and Lunt, 1967; Ackerson and Youngner, 1975; Dudeck *et al.*, 1983) and seashore paspalum (Leonard, 1983). Shoot Na^+ concentrations in bermudagrass and seashore paspalum have been reported

to increase under saline conditions, while K^+ concentrations declined (Dudeck *et al.*, 1983; Dudeck and Peacock, 1985). Two cultivars of seashore paspalum adjusted osmotically to saline stress by lowering leaf osmotic potentials (Peacock and Dudeck, 1985). This study was conducted to compare plant growth responses, ion relations, and osmotic adaptations of eleven C_4 turfgrasses to increasing levels of NaCl.

MATERIALS AND METHODS

The experiment was conducted in solution culture in a glasshouse. Eleven C_4 turfgrasses (Table 1) were planted from sprigs in 9 cm diameter by 6 cm deep plastic pots having coarse screen bottoms, and filled with coarse silica sand. Pots were suspended over tubs containing 12 L of a constantly aerated, modified Hoagland no. 2 solution (Hoagland and Arnon, 1950) in deionized water, in which 2 mg Fe L^{-1} was supplied with Fe-EDDHA chelate (Ciba-Geigy Sequestrene 138). Grasses were allowed to become densely established in pots before treatments were initiated. To avoid salinity shock, salinity levels were gradually increased by increments of 2.8 g NaCl L^{-1} (resulting in a solution concentration of 48 *mM* NaCl) every two days until final treatment levels of 0.7, 10, 20, and 30 dS m^{-1} electrical conductivity (0, 99, 198, and 298 *mM* NaCl) were reached. There were three replications per salinity level. Thereafter, growing solutions were kept at a constant volume with deionized water, and nutrient solutions changed weekly to maintain specified salinity levels.

Table 1. Turfgrasses evaluated for salinity tolerance.

Common name	Classification
Seashore paspalum (Hawaii Sel.)	*Paspalum vaginatum* Swartz
Floratine St. Augustinegrass	*Stenotaphrum secundatum* Walt.
St. Augustinegrass (Hawaii Sel.)	*Stenotaphrum secundatum* Walt.
Japanese lawngrass	*Zoysia japonica* Steud.
Manilagrass	*Zoysia matrella* (L.) Merr.
Commmon bermudagrass (Hawaii Sel. 1)	*Cynodon dactylon* (L.) Pers.
Commmon bermudagrass (Hawaii Sel. 2)	*Cynodon dactylon* (L.) Pers.
FB-137 bermudagrass	*Cynodon dactylon* (L.) Pers.
Sunturf bermudagrass	*Cynodon magennisii* (Hurcombe)
Tifgreen bermudagrass	*C. dactylon x C. transvaalensis* (Burtt-Davey)
Tifdwarf bermudagrass	*C. dactylon x C. transvaalensis* (Burtt-Davey)

Immediately after final treatment salinity levels were reached shoots and roots were clipped (shoots at 2.5 cm height, and roots at the bottom of pot screens) and the clippings discarded. Thereafter shoots were clipped twice at 10-day intervals using a cutting height of 2.5 cm (Tifdwarf, Sunturf, and manilagrass were cut at 2.0 cm). Both harvests were combined for subsequent analyses. Immediately prior to clipping, shoots were thoroughly rinsed for 20 seconds in deionized water, then clipped, blotted dry, and immediately put into tared, 120 ml glass bottles with air-tight snap-on lids for fresh weight determination. At the end of the experiment (20 days following initial clipping) roots growing through the screen were clipped and combined with those remaining within pots. Roots were rinsed in deionized water for 20 seconds, and blotted dry.

Shoots and roots were dried in a forced-air dryer at 70°C for 48 hours for dry weight determination, then ground in a Wiley mill with a 20-mesh screen and placed in air tight containers. Prior to ion analysis they were redried at 70°C, and 450 mg samples were ashed in a muffle furnace at 450°C for 7 hours. Ash was dissolved in 1 *M* HNO_3 for 5 hours, then diluted with deionized water and allowed to sit overnight, then shaken before aliquots were taken for analysis. Sodium and K^+ were determined by flame emission spectrophotometry and Cl^- with an Orion Cl^- ion activity electrode. All ions are expressed as mmol g^{-1} tissue dry weight.

Leaves for sap osmolality determination were rinsed as above, but allowed to dry on the plant before clipping. Two subsamples per pot were placed in 1.5 ml flexible plastic microcentrifuge tubes with air-

tight covers and frozen. Thawed tubes were flattened in a hydraulic press to crush tissue, releasing cellular sap. Sap osmolality was measured with a Wescor model 5100C vapor pressure osmometer.

Data were analyzed by regression, using an approach similar to that described by the SAS Institute as "testing for heterogeneity of slopes" (Freund *et al.*, 1986), except that testing was extended to quadratic effects. A more apt description for this analysis might be "testing for heterogeneity of linear and quadratic effects". In the regression model the continuous measured variable was salinity. However, there was also a qualitative, or indicator variable, grass, enabling the data to be stratified into groups, with different regression coefficients for linear and quadratic effects assigned to each grass. The regression model tested whether or not these regression coefficients were constant over groups (grasses). A model sequence approach was used for each response variable, the most general model including terms for common intercept, linear, and quadratic differences among grasses (Allen and Cady, 1982). Testing progressed until reduced models were found that described the data adequately. The overall goodness of fit of reduced models is described in figures presented by the model R^2. Single degree of freedom contrast coefficients were used to compare intercepts and regression coefficients among individual grasses.

As responses within the same genus were very similar (except in the Zoysieae) for variables other than shoot growth, bermudagrasses and St. Augustinegrasses were each grouped together before analysis. If two or more grasses were not significantly different as to intercept, slope,

or curvature, they were presented as a single regression equation. In all figures, labels for the grasses have been abbreviated to: Bers. (bermudagrasses), Japn. (Japanese lawngrass), Manl. (manilagrass), Pasp. (seashore paspalum), and Saugs. (St. Augustinegrasses).

RESULTS AND DISCUSSION

Growth Responses

Relative top growth, expressed as a percent of control decreased linearly with increasing salinity. This enabled grasses to be ranked for salinity tolerance according to relative magnitudes of the slopes of regression of relative top growth on salinity (Table 2). Based on this criteria, seashore paspalum, St. Augustinegrasses, and manilagrass were more salt tolerant than bermudagrasses, except for one selection of common bermudagrass. Both common bermudagrass selections were more salt tolerant than Tifgreen bermudagrass. Dudeck *et al.* (1983) reported common bermudagrass to be less salt tolerant than Tifgreen, Tifdwarf, and FB-137 bermudagrass. However, no mention was made of the source of the common selection. Common bermudagrass is genetically variable, and differences in salt tolerance between two common selections have been reported (Ramakrishnan and Nagpal, 1973). Common selections in this experiment were expected to be salt tolerant, as they were collected near the windward coast of Oahu.

Root growth increased under intermediate salinities, then decreased in bermudagrasses, manilagrass, and seashore paspalum, however this tendency was greatest in seashore paspalum (Fig. 1). Increased

Table 2. Ranking of turfgrasses according to slope of linear regression coefficients of relative shoot dry weight on salinity.

Grass	Slope
Seashore paspalum	-1.02 a[z]
Florantine St. Augustinegrass	-1.13 a
Hawaii Sel. St. Augustinegrass	-1.22 a
Manilagrass	-1.40 a
Hawaii Sel. 1 bermudagrass	-1.58 ab
Hawaii Sel. 2 bermudagrass	-1.65 b
Sunturf bermudagrass	-1.95 bc
Tifdwarf bermudagrass	-1.97 bc
FB-137 bermudagrass	-2.02 bc
Japanese lawngrass	-2.04 bc
Tifgreen bermudagrass	-2.20 c

[z]Slopes followed by the same letter are not significantly different at the 0.05 probability level as determined by single degree of freedom contrasts.

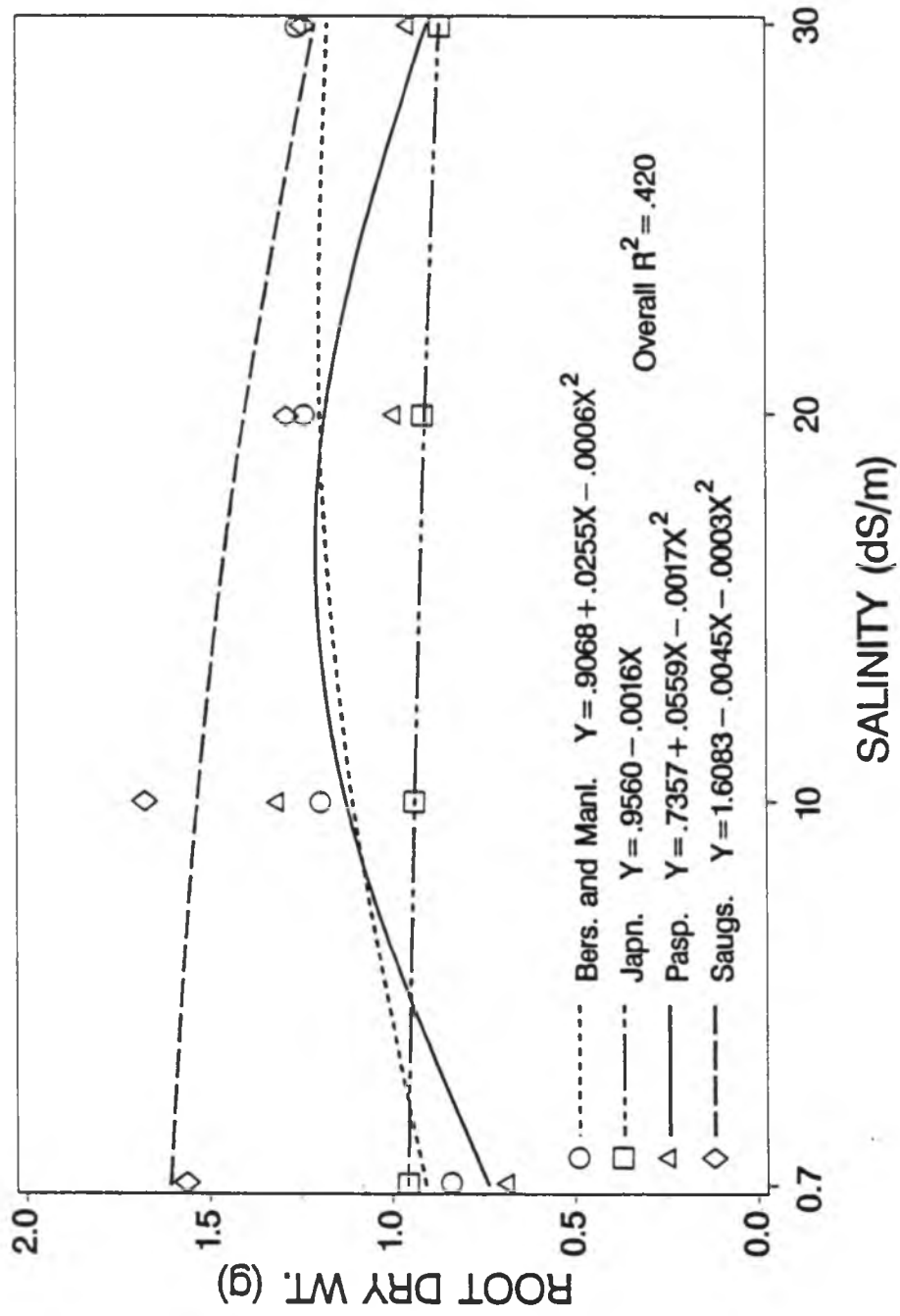


Figure 1. Root dry weight regrowth as influenced by NaCl level.

root growth under intermediate salinities has been reported previously for bermudagrass (Ackerson and Youngner, 1975) and seashore paspalum (Leonard, 1983), and may be an adaptation to salinity, resulting in more efficient water and nutrient uptake (Gorham *et al.*, 1985b). Root growth of St. Augustinegrasses and Japanese lawngrass declined under salinity, although St. Augustinegrasses had greater total root weights over all salinities than other grasses.

Tissue Ion Concentrations

Sodium and Cl^- concentrations were low under salt stress in the shoots of bermudagrasses and manilagrass, remaining less than 0.5 mmol g^{-1} Na^+ and 0.4 mmol g^{-1} Cl^-, respectively (Figs. 2 and 3). In seashore paspalum and Japanese lawngrass Na^+ concentrations of 1.2 mmol g^{-1} and Cl^- concentrations of about 0.7 mmol g^{-1} were reached at 30 dS m^{-1} salinity. St. Augustinegrasses accumulated Na^+ and Cl^- in the shoots to 2.1 and 1.3 mmol g^{-1}, respectively. These levels are four to five times higher than the bermudagrasses and manilagrass, and are similar to the levels reported in some salt accumulating halophytes (Gorham *et al.*, 1980; Storey *et al.*, 1977).

Root Na^+ and Cl^- followed the same trends as in the shoots, St. Augustinegrasses accumulating to high levels (Figs. 4 and 5). Under saline conditions, Na^+ and Cl^- were maintained at lower concentrations in the shoots than the roots in manilagrass, indicating a partial restriction of Na^+ and Cl^- accumulation in the shoots. In bermudagrasses Na^+, but not Cl^-, was lower in shoots relative to roots. In contrast, shoot Na^+ and Cl^- concentrations were higher than root

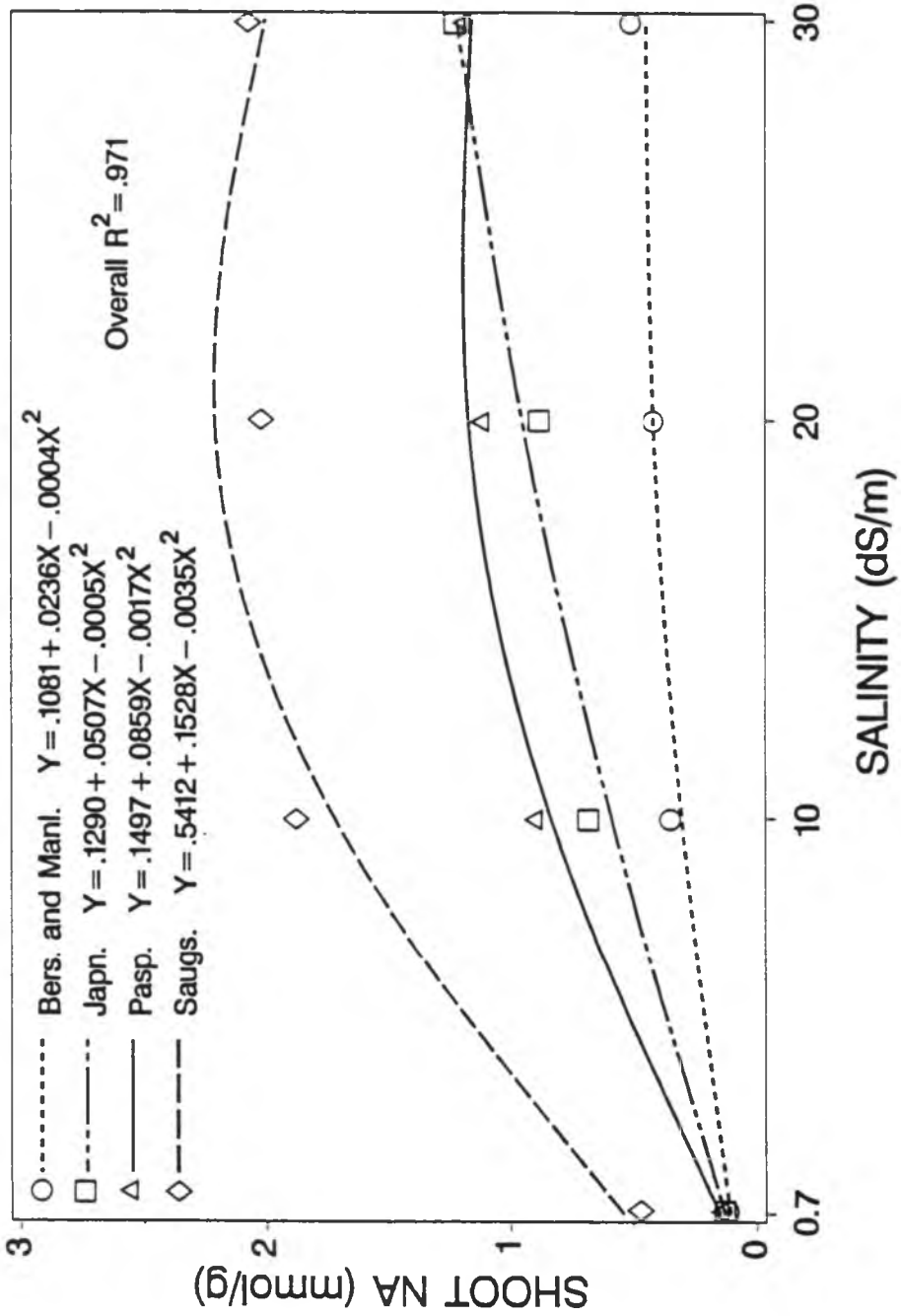


Figure 2. Shoot Na^+ concentration, expressed on a tissue dry weight basis, as influenced by NaCl level.

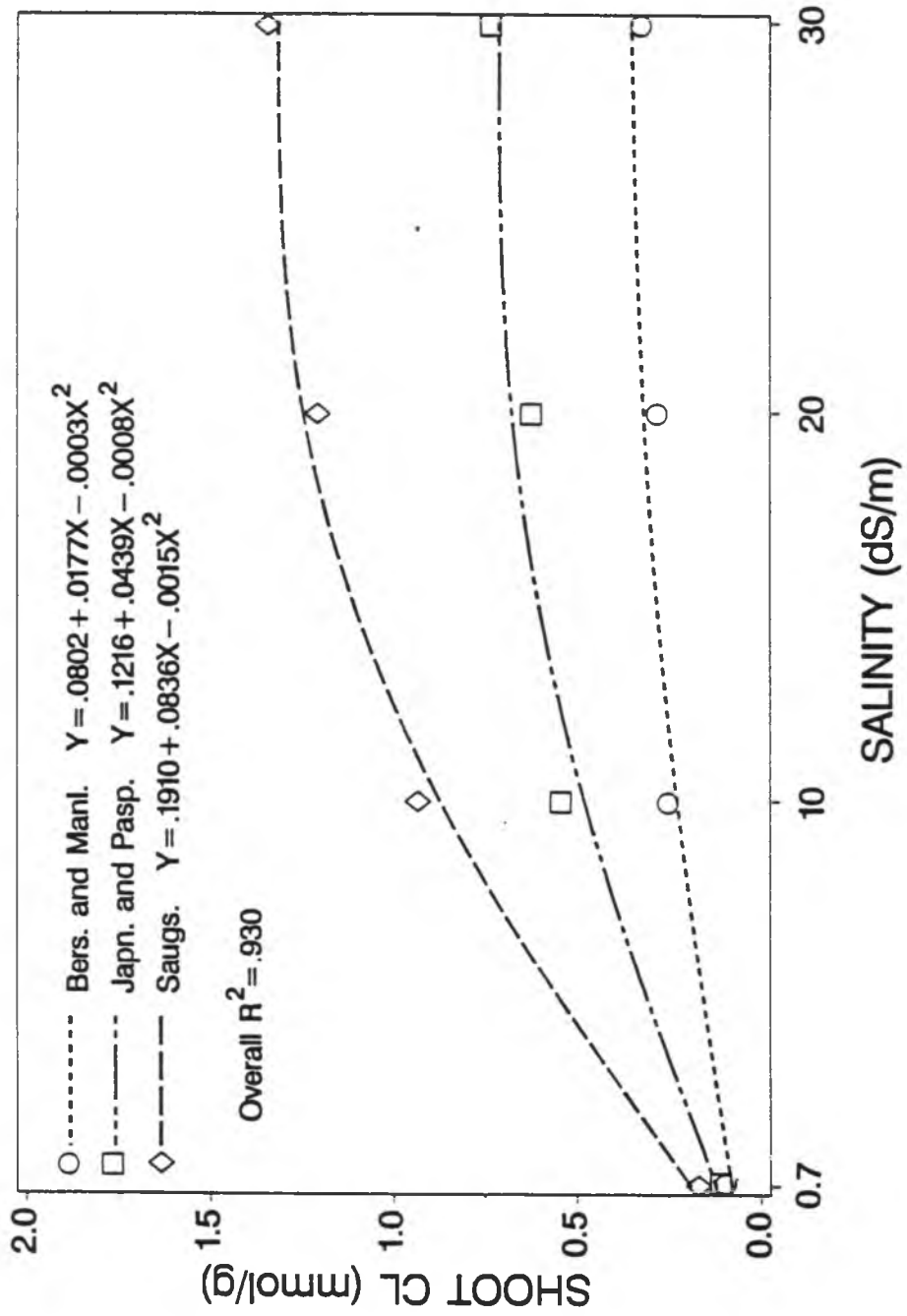


Figure 3. Shoot Cl^- concentration, expressed on a tissue dry weight basis, as influenced by NaCl level.

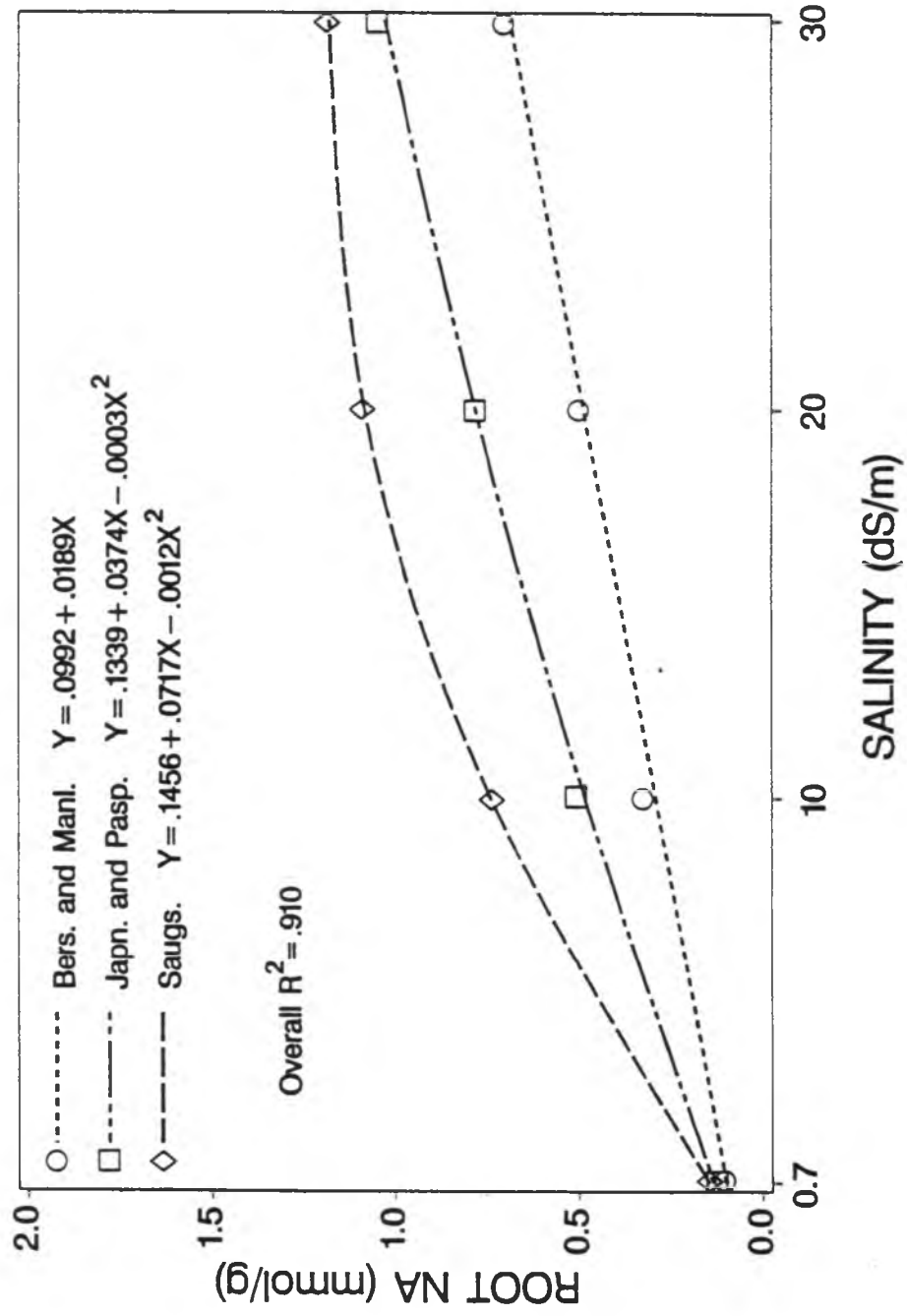


Figure 4. Root Na^+ concentration, expressed on a tissue dry weight basis, as influenced by NaCl level.

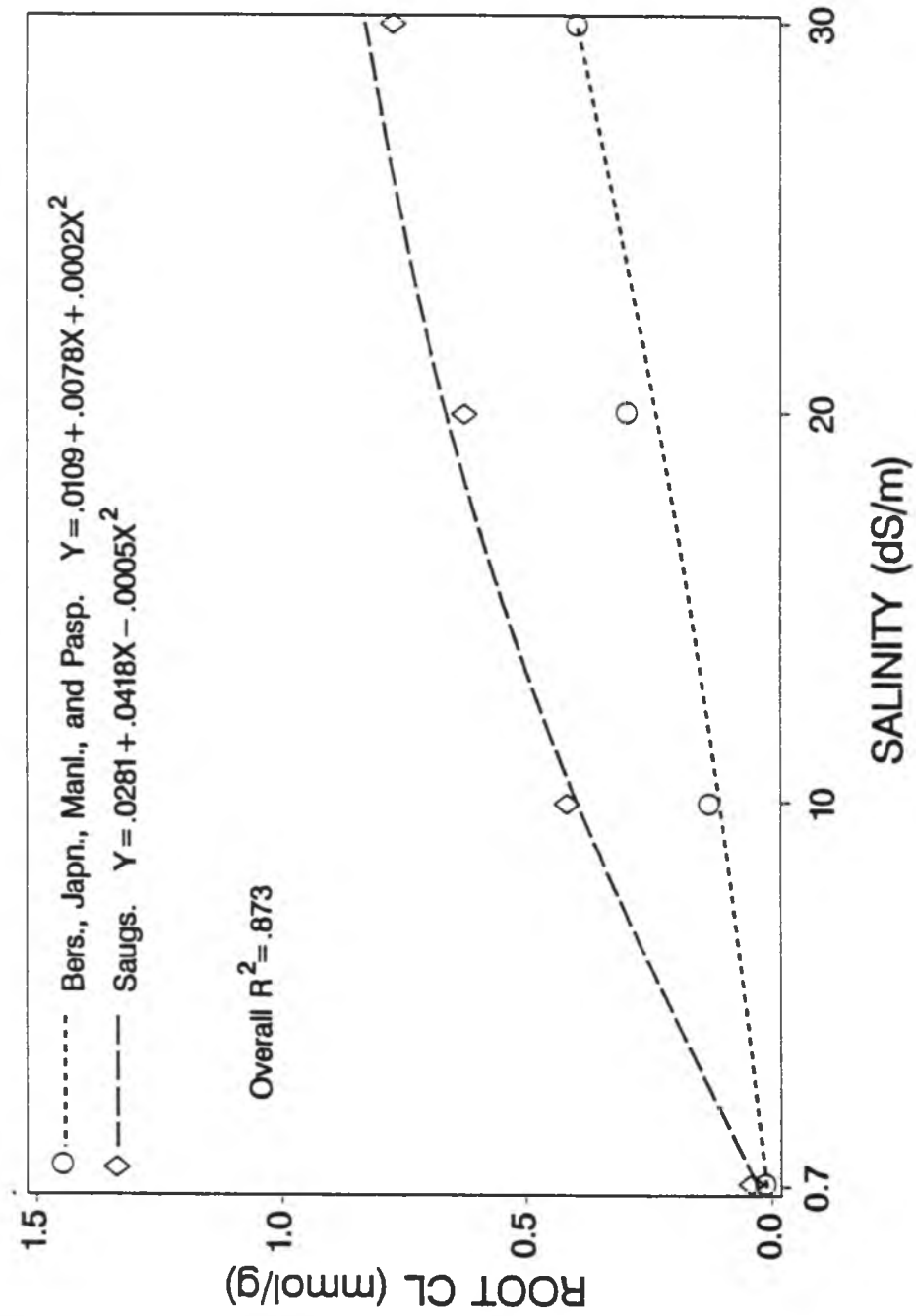


Figure 5. Root Cl^- concentration, expressed on a tissue dry weight basis, as influenced by NaCl level.

concentrations in seashore paspalum and Japanese lawngrass, and much higher in St. Augustinegrasses. Sodium and Cl^- may be restricted from shoots by selective root K^+ uptake/Na^+ exclusion or by special epidermal leaf glands that secrete salt (Kramer, 1984). Leaf salt glands which selectively secrete Na^+ and Cl^- have been reported in bermudagrass (Liphschitz and Waisel, 1974), and have recently been found in manilagrass and Japanese lawngrass of the tribe Zoysieae (Marcum and Murdoch, 1989). Glands in Japanese lawngrass are much less efficient, secreting much less salt than those in manilagrass.

Salt tolerance has been associated with high shoot K^+ concentrations in relation to Na^+ and Cl^- in barley (Storey and Wyn Jones, 1978b), and tall wheatgrass (Shannon, 1978). Seashore paspalum maintained higher shoot and root K^+ under salt stress than did the other grasses (Figs. 6 and 7). Bermudagrasses and manilagrass maintained relatively stable K^+ levels with increasing salinity. Shoot K^+/Na^+ ratios dropped in all grasses due to increasing salinity, but in bermudagrasses the ratio remained greater than one at high salinity, indicating shoot selectivity for K^+ over Na^+.

Osmotic And Water Relations

Shoot sap osmolality increased in all grasses with increasing salinity, and was maintained above the levels of the growing solution, indicating that the grasses adjusted osmotically under increasing salinity (Fig. 8). However, shoot tissue water contents decreased with increasing salinity, indicating that osmotic adjustment was not achieved exclusively by solute accumulation, but also by tissue dehydration

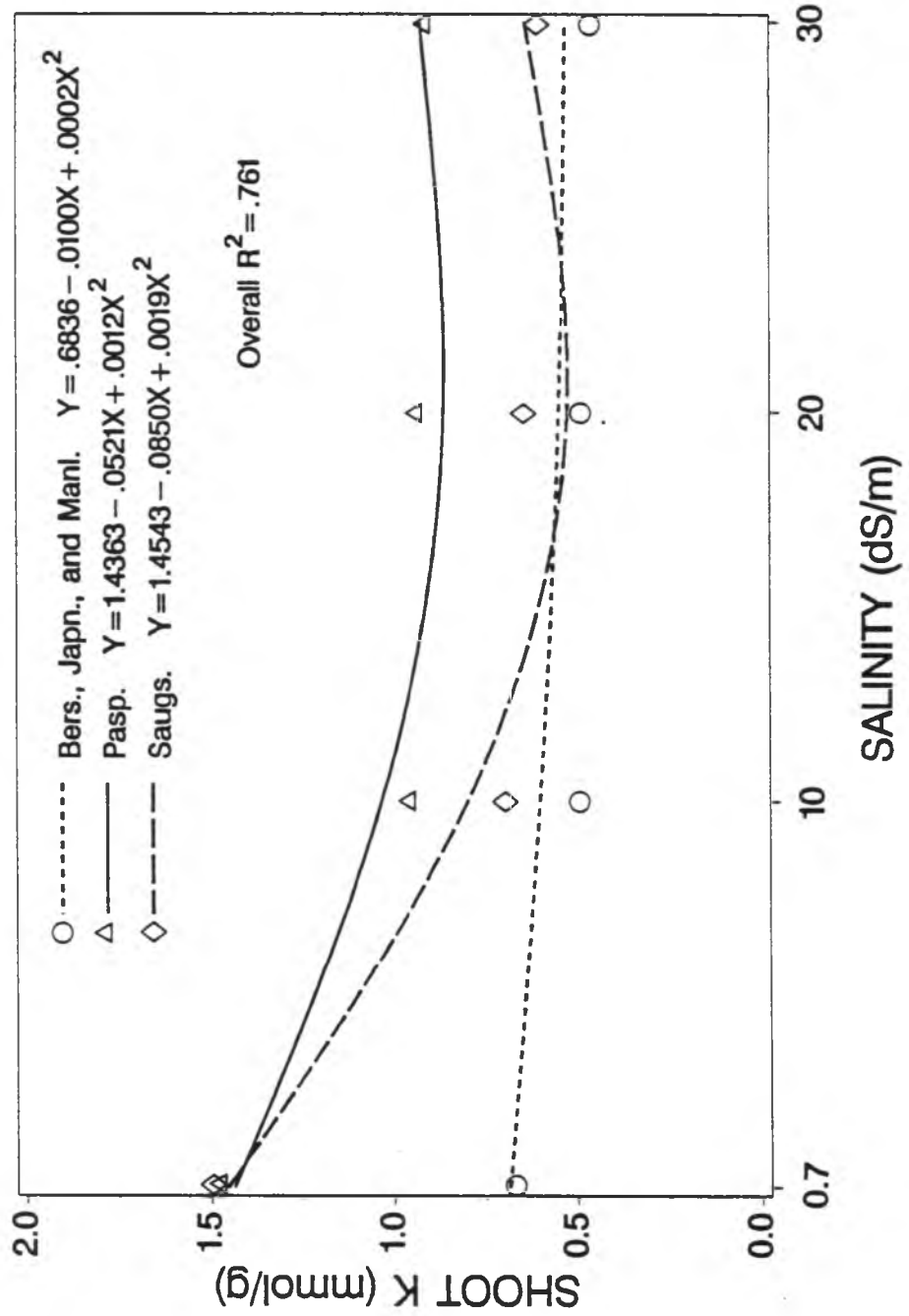


Figure 6. Shoot K^+ concentration, expressed on a tissue dry weight basis, as influenced by NaCl level.

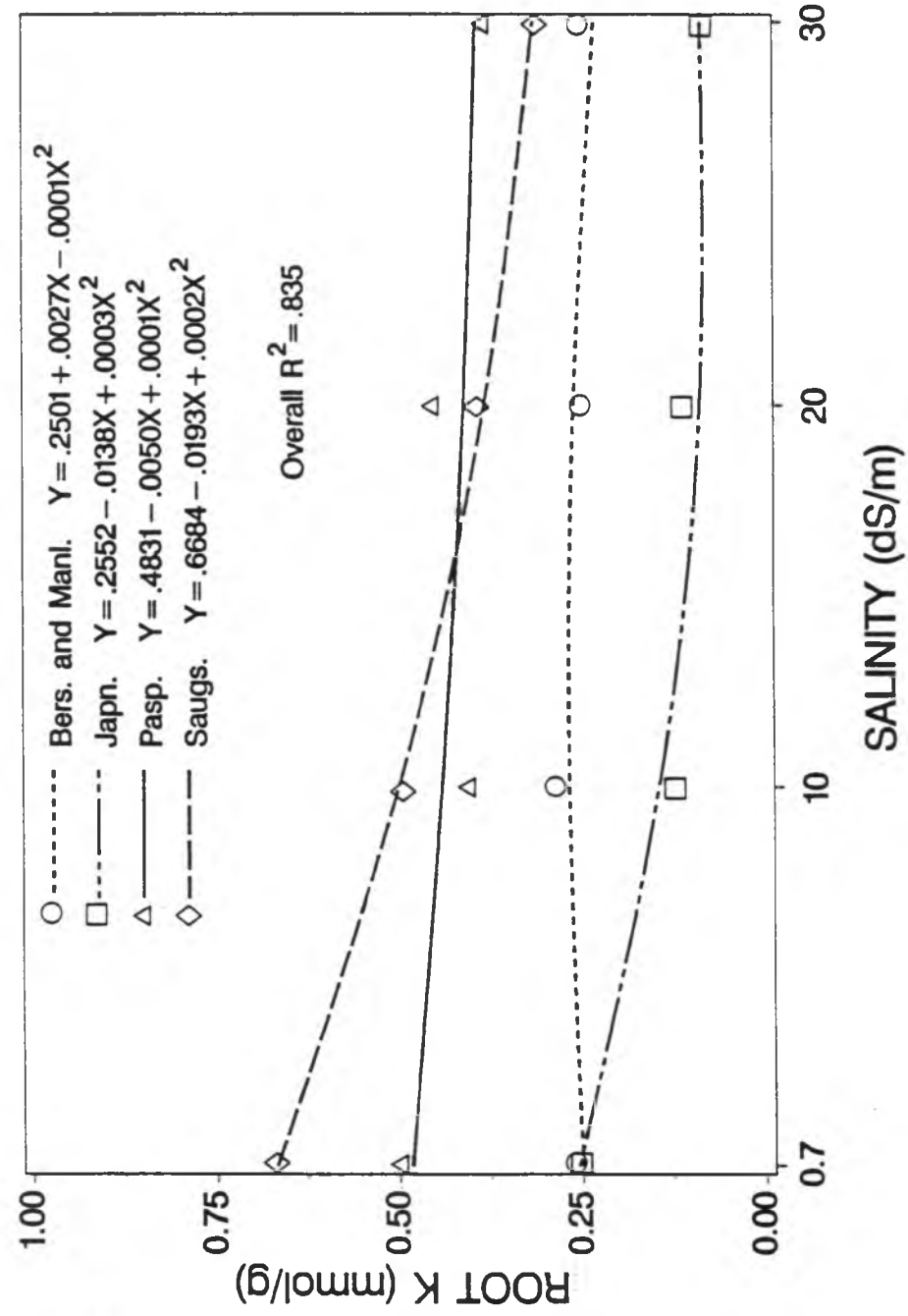


Figure 7. Root K^+ concentration, expressed on a tissue dry weight basis, as influenced by NaCl level.

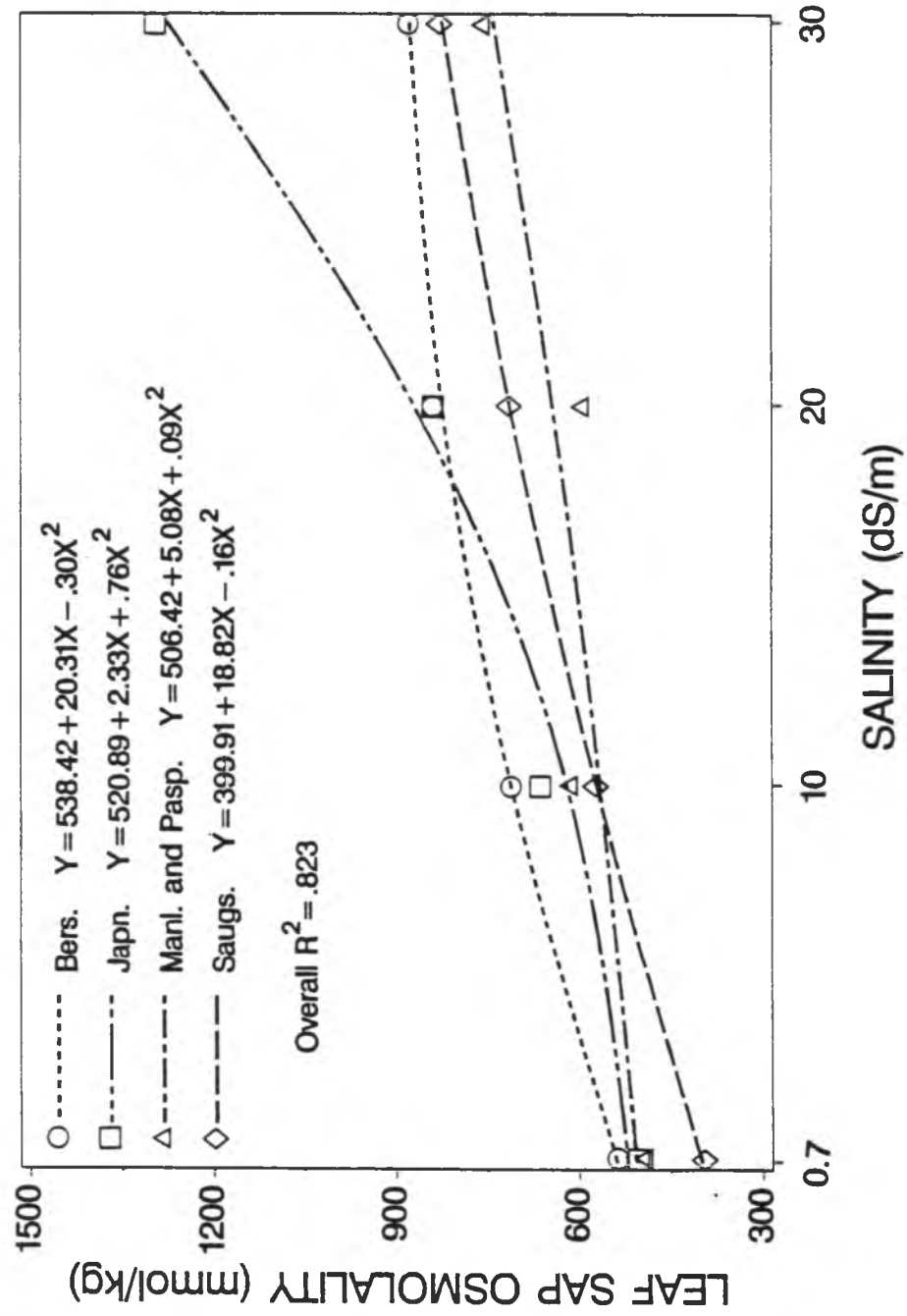


Figure 8. Leaf sap osmolality as influenced by NaCl level.

(Fig. 9). Shoot dehydration is a typical response of Poaceae to salt stress, indicating water stress due to water imbalance which results in reduced growth, as growth is intimately tied to maintenance of cell turgor (Hellebust, 1976). Seashore paspalum and St. Augustinegrasses, members of the subfamily Panicoideae (Gould and Shaw, 1983), maintained much higher tissue water levels than did bermudagrasses, manilagrass, and Japanese lawngrass (Chloridoideae subfamily), which may be responsible for their higher growth rates under salt stress. Leaf sap osmolalities reached very high levels, up to 1290 mOsmol kg^{-1} (cf. sea water approximately 1000 mOsmol kg^{-1}) in Japanese lawngrass. This was due to relatively high shoot Na^+ and Cl^+ concentrations (expressed on a dry weight basis) concurrent with very low shoot tissue water levels. In contrast, St. Augustinegrasses, although having extremely high shoot Na^+ and Cl^- concentrations, maintained very high shoot tissue water levels, which resulted in intermediate sap osmolalities.

In summary, there are wide differences in responses to salinity among the grasses studied, and particularly between the two subfamilies represented. Bermudagrasses and manilagrass could be classified as "ion regulators", maintaining shoot Na^+ and Cl^- at low levels, while concurrently maintaining high shoot K^+/Na^+ ratios, under salt stress. Both grasses have highly active and selective salt glands. Other grasses accumulated Na^+ and Cl^- to much higher levels. However, St. Augustinegrasses and seashore paspalum were able to avoid high sap ion concentrations (ie. maintained relatively low leaf sap osmolalities) by maintaining high shoot tissue water levels under salt stress.

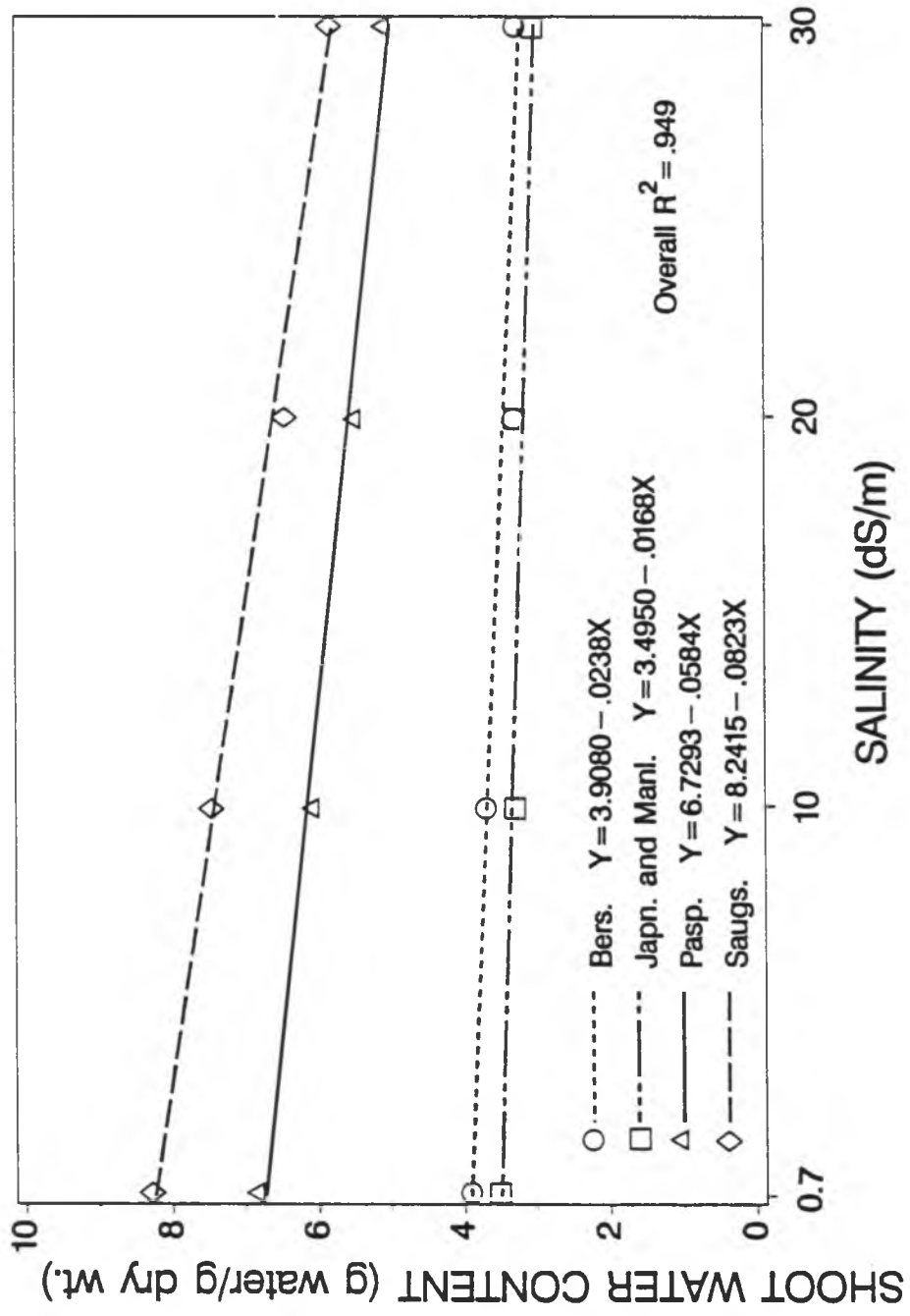


Figure 9. Shoot tissue water content as influenced by NaCl level.

CHAPTER IV

SALT GLANDS IN THE ZOYSIEAE

ABSTRACT

During salinity tolerance experiments, salt crystals were observed on the leaves of *Zoysia matrella* (L.) Merr. and *Zoysia japonica* Steud. of the tribe Zoysieae, subfamily Chloridoideae (Poaceae). As salt crystals are indicative of active salt secretion and salt glands have not been reported in the Zoysieae, a search was initiated for salt glands on these two species using scanning electron microscopy. Salt glands were found in both species. Glands protrude from and are recumbent to the leaf epidermis and consist of a basal cell and upper cap cell. Glands were better developed on the adaxial surfaces, while those on the abaxial surfaces appeared to be nonfunctional. The more salt tolerant species, *Z. matrella*, had a higher density of larger glands and secreted more sodium per unit leaf mass, resulting in much lower leaf sodium concentrations and sap osmolalities than those of *Z. japonica*. The finding of salt glands in the tribe Zoysieae confirms its relation to the four other tribes within the subfamily Chloridoideae in which salt glands have previously been reported.

INTRODUCTION

In vitro studies have revealed that enzymes from both halophytes and glycophytes have similar sensitivities to salt (Greenway and Munns,

1980). Salt tolerant plants must strictly limit concentrations of saline ions in cytoplasm to avoid enzyme deactivation (Flowers *et al.*, 1977; Gorham *et al.*, 1985b). Plants may regulate ion concentrations by a number of means: intracellular compartmentation of Na^+ and Cl^- in vacuoles (Wyn Jones, 1981), Na^+ and Cl^- exclusion or Na^+/K^+ exchange at the root cortex (Greenway and Munns, 1980), Na^+ reabsorption by xylem parenchyma cells and export from the root (Yeo *et al.*, 1977), phloem reabsorption of Na^+ and export (Flowers *et al.*, 1977), redistribution of saline ions to senescing leaves (Yeo and Flowers, 1984), and elimination of excess saline ions by salt glands or bladders (Flowers *et al.*, 1977).

Specialized multicellular epidermal salt glands are present in several families of dicotlyledons, such as Frankeniaceae, Plumbaginaceae, Tamaricaceae, and others (Fahn, 1988). Within the Poaceae, salt glands were first reported in the halophytic genera *Spartina* (Skelding and Winterbotham, 1939), and later in *Aeluropus* (Waisel, 1972). At present, salt glands have been found in over 30 grass species of the tribes Chlorideae, Eragrosteae, Aeluropodeae, and Pappophoreae (Lipshchitz and Waisel, 1974; Leonard, 1983; Taliesnik and Anton, 1988), all members of the subfamily Chloridoideae (Gould and Shaw, 1983).

Salt glands of the Poaceae appear to be modified trichomes, consisting of two cells, a basal and cap cell. The position, size, and shape of the glands varies between species (Liphshitz and Waisel, 1974). Although salt glands in *Spartina townsendii* H. and J. Groves, a grass halophyte, have been studied in depth, the mechanism of salt secretion is not yet known (Thomson and Healey, 1984).

During salinity experiments, salt crystals were observed on the leaves of *Zoysia matrella* (L.) Merr. and *Zoysia japonica* Steud., of the tribe Zoysieae. As salt crystals are indicative of active salt secretion, a search was initiated for salt glands on these two species.

MATERIALS AND METHODS

Growing Conditions

Plants were started from rhizomes in 9 cm diameter, sand-filled plastic pots having coarse screen bottoms. Pots were suspended over 12 L tubs of a modified, 1/2 strength Hoagland no. 2 solution (Hoagland and Arnon, 1950) in which 2 ppm Fe^{3+} was supplied as an Fe-EDDHA chelate. Solutions were constantly aerified and maintained at constant volume. Salt treatment levels were achieved by addition of 50 *mM* increments of NaCl every 2 days until final levels of 0, 100, 200, and 300 *mM* were reached. There were three replications. Thereafter, growing solutions were changed weekly to maintain specified salinity levels. Leaves were thoroughly rinsed with deionized water weekly throughout the experiment to avoid excessive accumulation of salt crystals. Grasses were clipped weekly at a height of 2.5 cm throughout the experiment. Leaves for analysis were cut four weeks after final salinity levels were reached to allow plants to fully equilibrate to the different salinity levels.

Ion Contents

Leaves for ion analysis were dried at 70°C, then ashed at 450°C for 7 hours. Ash was dissolved in 1 *N* HNO3, then diluted in deionized

water. Sodium and K^+ were determined by flame emission spectrophotometry and Ca^{2+} and Mg^{2+} by atomic absorption spectrophotometry. Ions were determined in both unrinsed and rinsed tleaves. For the latter, leaves were gently rinsed in deionized water for 20 seconds, which was sufficient to remove deposited salt.

Sap Osmolality

Leaves were thoroughly rinsed with deionized water and allowed to dry before clipping, then placed in 1.5 mL plastic microcentrifuge tubes with air-tight covers and frozen on dry ice. Two subsamples were taken per pot. Thawed tubes were flattened an a hydraulic press to release leaf sap. Sap osmolality was measured with a Wescor 5100C vapor pressure osmometer.

Electron Microscopy

Leaf segments 2 to 5 mm long were fixed in 2.5% gluteraldehyde in 0.1 *M* phosphate buffer (pH 7.0) for 1 hour, rinsed 5-10 minutes in phosphate buffer, then postfixed in 1% phosphate buffered OsO_4. Specimens were dehydrated in a graded ethanol series, and critical point dried using liquid CO_2. Mounted specimens were sputter-coated with gold-paladium, and observed with a Cambridge Stereoscan S-150 scanning electron microscope operated at 10 KeV.

RESULTS AND DISCUSSION

Secreted salt crystals were observed on the adaxial surfaces only of the leaves of both *Zoysia matrella* and *Zoysia japonica* (Fig. 10). This would indicate that glands on the abaxial surface, though present, are inactive. In contrast, we have observed salt crystals on both the adaxial and abaxial leaf surfaces of *Cynodon dactylon* (L.) Pers. and *Sporobolus virginicus* (L.) Kunth.

In both *Zoysia* species, salt glands protrude out from the leaf surface, and are clearly discernable in longitudinal rows parallel to the veins and between rows of stomata (Fig. 11). The glands are recumbent to the leaf surface, much like those of the genus *Bouteloua* and *Buchloe* (Liphschitz and Waisel, 1974), and have a basal and cap cell. The basal cells are cutinized in both species, but to a greater extent in *Z. matrella* (Fig. 12). Glands in both species are surrounded by numerous papillae. Salt glands are approximately 3 times more numerous in *Z. matrella* than in *Z. japonica* and are also larger in size, being longer, but of the same width (Table 3). Glands are also present on the abaxial leaf surfaces (Fig. 13), but are smaller and do not appear to function.

Sodium, K^+, Ca^{2+} and Mg^{2+} were determined in rinsed and unrinsed leaves of plants grown in 200 *mM* NaCl (Table 4). The total Na^+ of unrinsed leaves (tissue Na^+ plus secreted Na^+) was similar for both species, but tissue Na^+ (rinsed leaves) in *Z. japonica* was more than twice that of *Z. matrella*. This was due to a more efficient secretion of Na^+ by *Z. matrella*, secreting more than twice the Na^+ per unit leaf

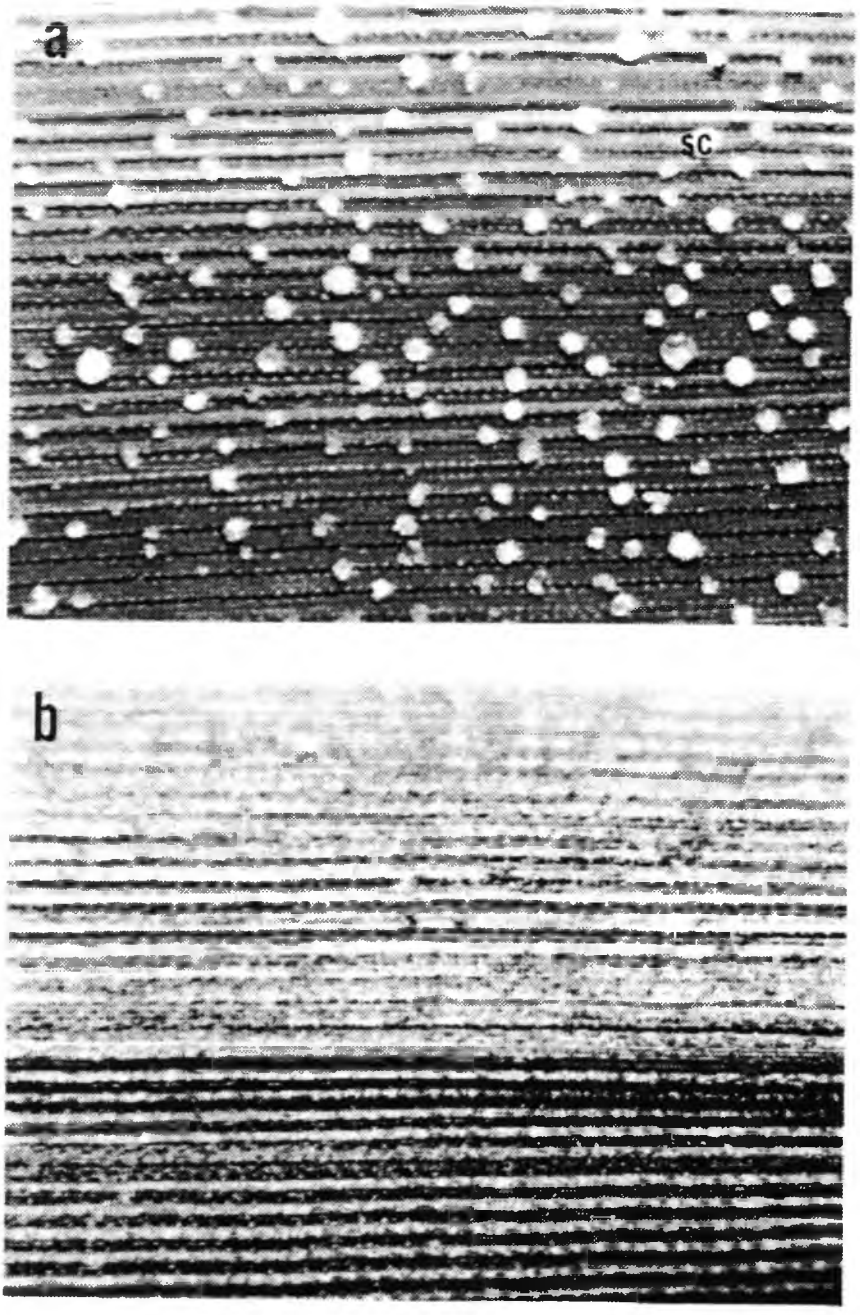


Figure 10. Photomicrograph (32X) of a) adaxial and b) abaxial leaf surfaces of *Zoysia japonoca*. SC=salt crystal.

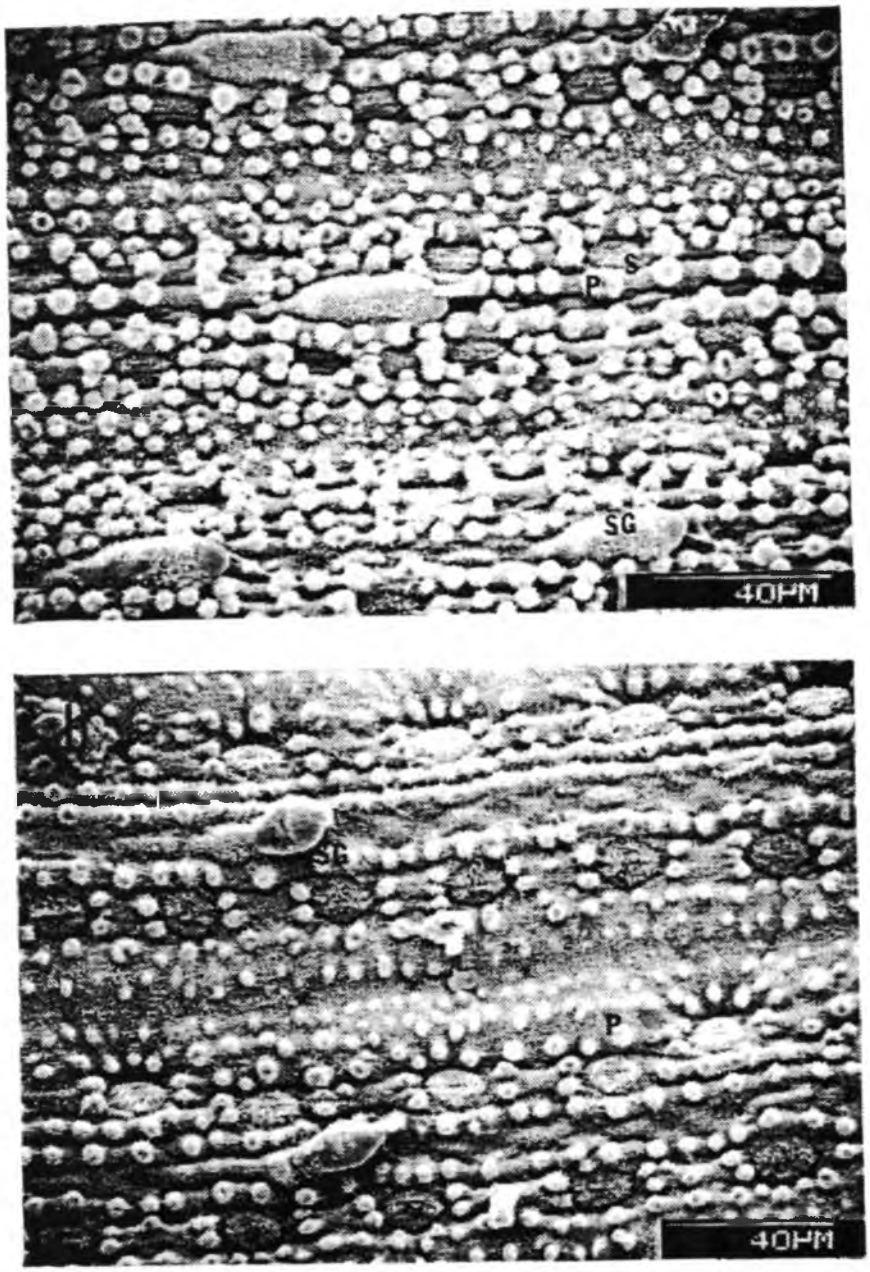


Figure 11. Scanning electron micrographs (587X) of adaxial leaf surfaces of a)*Zoysia matrella* and b)*Z. japonica*. S=stomate, P=pappilla, SG=salt gland.

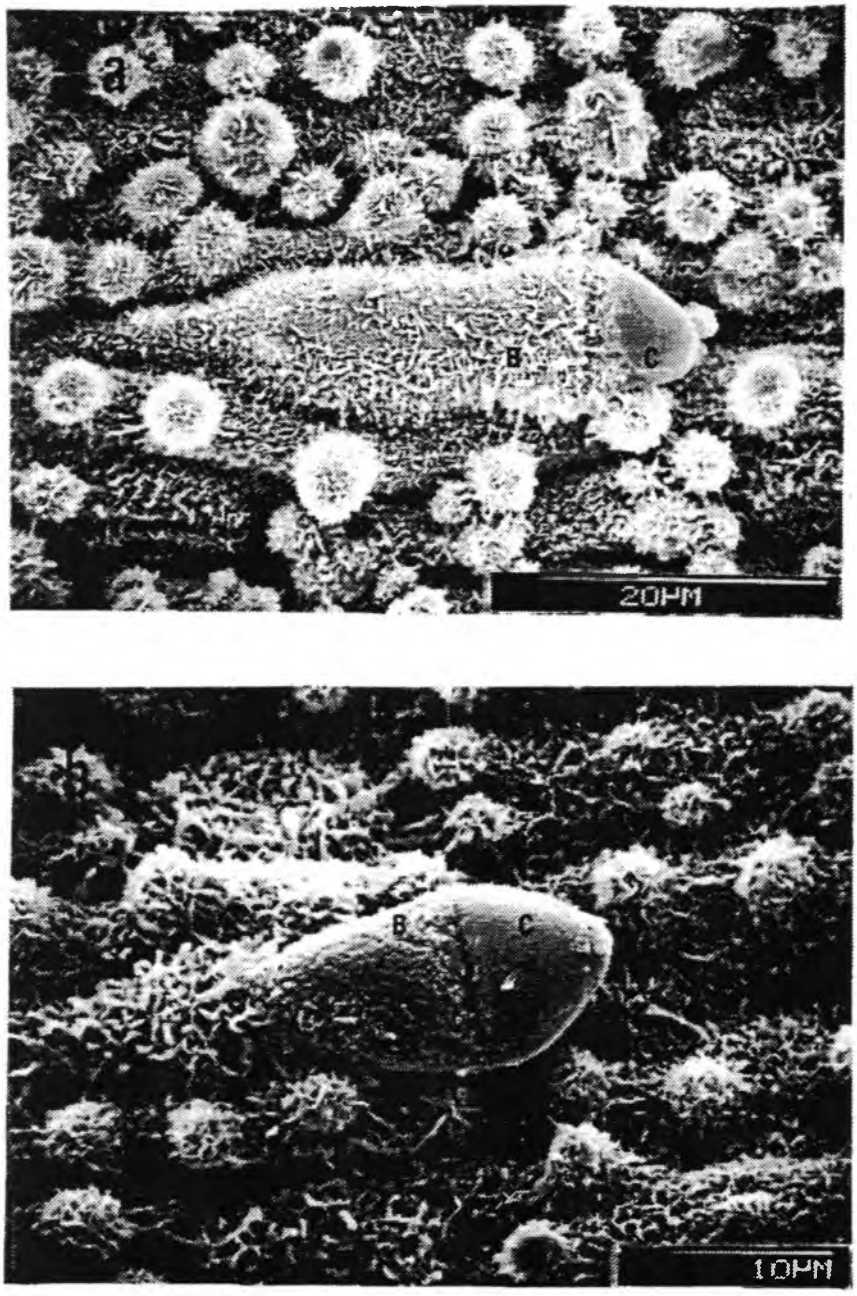


Figure 12. Scanning electron micrographs of salt glands on adaxial leaf surfaces of a)*Zoysia matrella* (1900X) and b)*Z. japonica* (2200X). B=basal cell, C=cap cell.

Table 3. Number and size of salt glands on the adaxial surface of leaves of *Zoysia matrella* and *Z. japonica* grown in nutrient solutions containing 100 *mM* NaCl.

Grass	No. Glands mm^{-2}[y]	Gland Size[z]	
		Length (μm)	Width Cap Cell (μm)
Zoysia matrella	75.9 ± 4.3	35.2 ± 0.77	12.7 ± 0.40
Zoysia japonica	27.6 ± 1.5	20.5 ± 0.47	12.4 ± 0.27

[z]Means ± s.e. of 7 measurements
[y]Means ± s.e. of 10 measurements

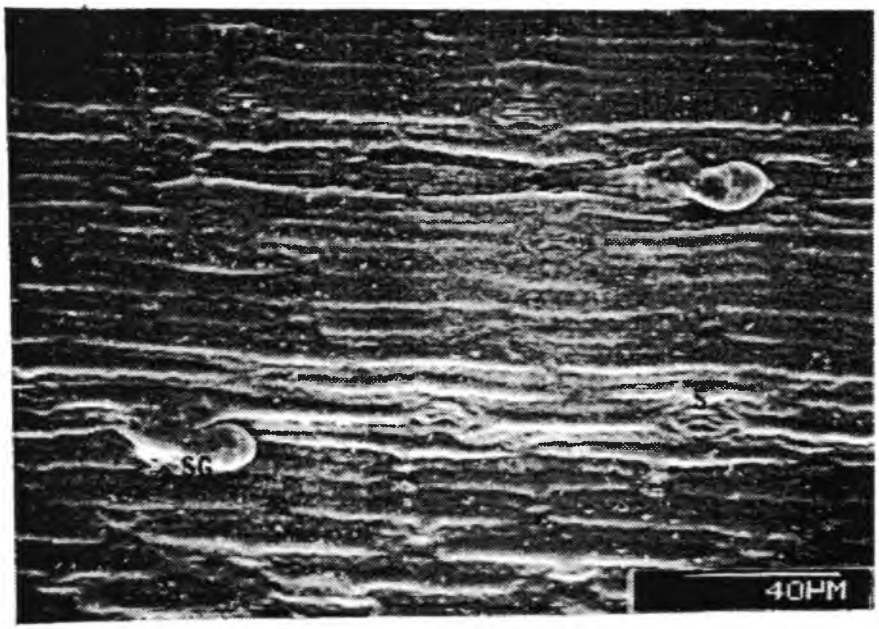


Figure 13. Scanning electron micrograph of abaxial leaf surface of *Zoysia japonica* (587X). S=stomate, SG=inactive salt gland.

Table 4. Concentration of Na^+, K^+, Ca^{2+}, and Mg^{2+} in unrinsed and rinsed leaves of *Zoysia matrella* and *Z. japonica* grown in nutrient solutions containing 200 *mM* NaCl. Differences between unrinsed and rinsed leaves represent salt secretion for one week.

	Ion							
	Na^+		K^+		Ca^{2+}		Mg^{2+}	
	matr.	*japn.*	*matr.*	*japn.*	*matr.*	*japn.*	*matr.*	*japn.*
Treatment	mmol g^{-1} dry weight							
Unrinsed	1.06	1.05	0.29	0.29	0.03	0.03	0.04	0.04
Rinsed	0.33	0.75	0.24	0.23	0.03	0.03	0.04	0.04
	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

*Means in columns are significantly different ($P<0.05$) as determined by Student's t test.

mass than did *Z. japonica*. These differences in Na^+ concentration confirmed observations that salt crystals were much more dense on the leaves of *Z. matrella*. Salt glands on the adaxial surface of leaves of *Z. matrella* were approximately 3 times more numerous and larger than those of *Z. japonica*, which would account for the differences in ion secretion observed. Potassium concentration was greater in unwashed leaves of both species, although not statistically significant, indicating that there a small amount of K^+ secretion may have occured.

In general, salt tolerance among closely related grasses has been associated with salt exclusion from shoots (Hannon and Barber, 1972; Yeo, 1983). *Z. japonica* was not able to regulate shoot tissue salt levels as efficiently as *Z. matrella*, resulting in very high shoot sap osmolalities under salt stress (Fig. 14). A sap osmolality of 1200 mOsmol kg^{-1} was reached in *Z. japonica* grown in 300 *mM* NaCl (osmolality in the growing medium approximately 600 mOsmol kg^{-1}). Sap osmolality for *Z. matrella* at the same salinity level was only 780 mOsmol kg^{-1}. Top growth reduction of *Z. japonica* grown at 300 *mM* NaCl was approximately 88% that of plants in unsalinized media while that of *Z. matrella* was approximately 27%. It is evident that salt tolerance of *Z. matrella* is related to efficient salt exclusion from leaves, due in part to efficient secretion through a large number of salt glands. Similar findings have been reported for two grasses in the genus *Pappophorum* in which greater tolerance to salinity of one species was related to more efficient ion secretion by salt glands (Taleisnik and Anton, 1988).

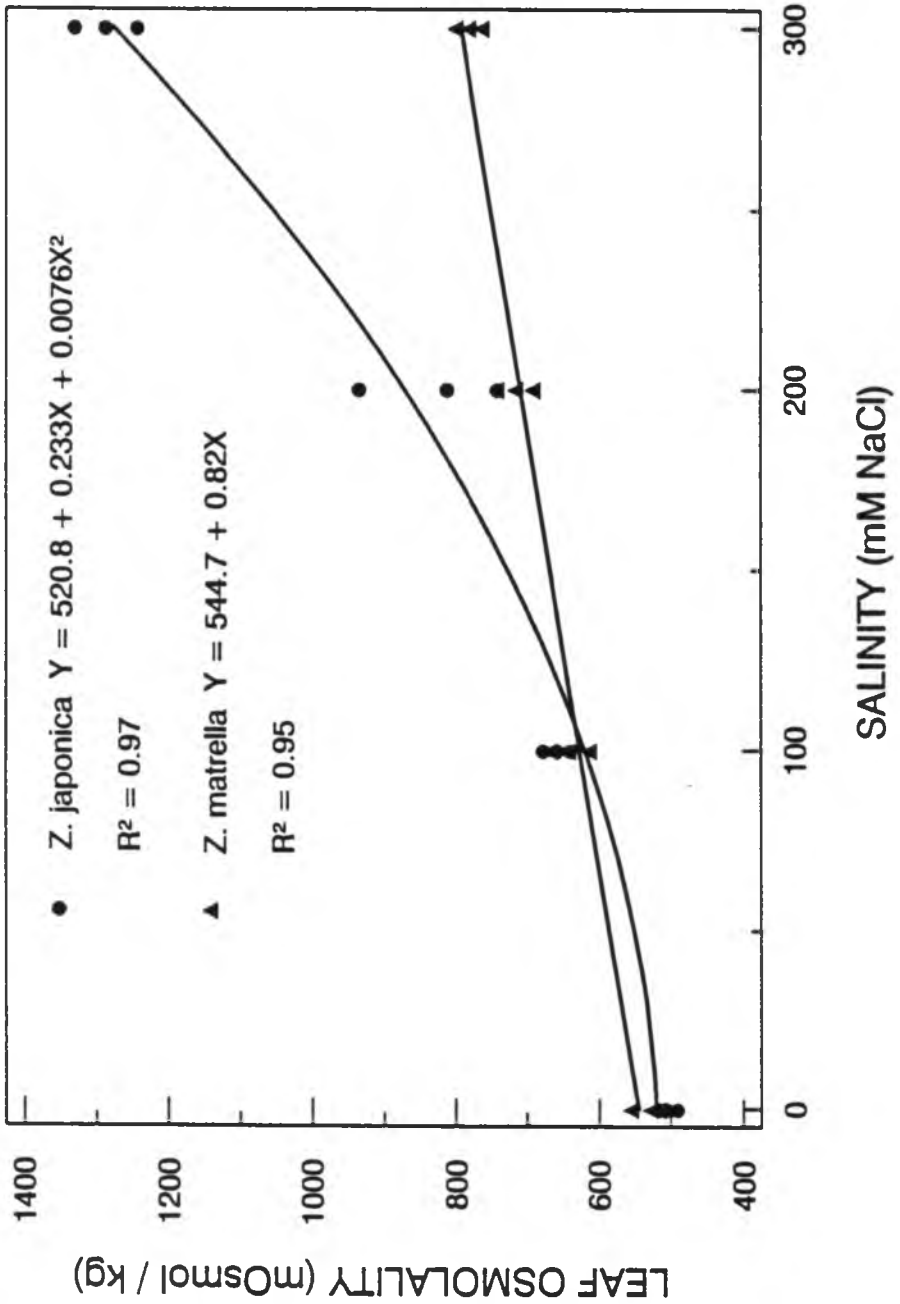


Figure 14. Leaf sap osmolalities of *Zoysia matrella* and *Zoysia japonica* as affected by increasing salinity of the growing media.

This is the first report of salt glands in the tribe Zoysieae and confirms its relationship to the four other tribes within the subfamily Chloridoideae in which salt glands have been reported (Chlorideae, Eragrosteae, Aeluropodeae, and Pappophoreae). This may indicate that, though most of these grasses cannot be considered halophytes, they all evolved from a common halophytic ancestor.

CHAPTER V

GROWTH AND PHYSIOLOGICAL RESPONSES OF 6 C_4 TURFGRASSES TO SALINITY

ABSTRACT

There is a need for salt tolerant turfgrasses in Hawaii and other areas where saline water is frequently used for irrigating turf. The objectives of this study were to compare the relative salt tolerance, growth, and physiological responses of 6 C_4 turfgrasses to salinity in an attempt to elucidate salinity tolerance mechanisms of these grasses. Grasses were grown in solution culture containing NaCl at concentrations of 1, 100, 200, 300, and 400 *mM*. Salinity tolerance was assesed both on the basis of reductions of relative shoot growth and turf quality with increasing salinity. St. Augustinegrass *(Stenotaphrum secondatum* Walt.), seashore paspalum (*Paspalum vaginatum* Swartz) , and manilagrass *(Zoysia matrella* (L.) Merr.) were very salt tolerant, maintaining high shoot growth rates and good turf quality at high salinity. Tifway bermudagrass *(Cynodon dactylon X C. transvaalensis* Burtt-Davey) was intermediate in salt tolerance, while Japanese lawngrass *(Zoysia japonica* Steud) and centipedegrass *(Eremochloa ophuriodes* (Munro) Hack.) were sensitive to salinity, suffering severe shoot dieback at intermediate, and low salinity, respectively. All grasses adjusted osmotically primarily by an increase in shoot Na^+ and Cl^- concentrations, though shoot dehydration also occured. In grasses other than St. Augustinegrass, salinity tolerance was related to exclusion of Na^+ and Cl^- from the shoots, coupled with shoot selectivity for K^+ over

Na^+. Seashore paspalum relied solely on the selective ion uptake/exchange mechanisms of the root cortex and endodermis, while in manilagrass and Tifway bermudagrass shoot Na^+ and Cl^- exclusion was aided by the presence of very active leaf salt glands. Sodium and Cl^- toxicity was evident in centipedegrass and Japanese lawngrass, which suffered severe leaf burn and shoot dieback at low to intermediate salinities. Shoot Na^+ and Cl^- levels were high at intermediate salinities, particularly if expressed on a tissue water basis. Responses of St. Augustinegrass to salinity were similar to those reported to occur in dicotyledonous halophytes. A significant stimulation of shoot growth rate occured under intermediate salinities, and was associated with shoot Na^+ and Cl^- accumulation and high shoot tissue water contents. It is generally accepted that salinity tolerance at the cellular level involves active compartmentation of Na+ and Cl- within the vacuoles, coupled with accumulation within the cytoplasm of nontoxic compatible solutes, the primary candidates in the Poaceae being glycinebetaine and proline (Wyn Jones, 1981). Levels of glycinebetaine and proline increased in the shoots of all grasses except in centipedegrass, which had very low levels of both compounds. Assuming that glycinebetaine and proline are located exclusively in the cytoplasm, which makes up about 10% of the total cell volume, they would make a significant contribution to the osmotic adjustment of the cytoplasm at intermediate to high salinity in all grasses except centipedegrass.

INTRODUCTION

The use of brackish groundwater or saline sewage effluent water in many areas for irrigation has resulted in a need for salt tolerant turfgrasses. Overuse of limited water resources in Hawaii and Florida has resulted in sea water contamination of fresh water wells, some of which are used for turfgrass irrigation (Adams, 1978; Murdoch, 1987).

Responses and adaptations of plants to salinity have been reviewed (Gorham *et al.*, 1985b; Yeo, 1983; Cheeseman, 1988.). The detrimental effects of salinity on plant growth are due either to the indirect effect of saline ions on the water potential of the soil, resulting in osmotic imbalances and turgor loss within the plant, or to the direct effects of ion toxicity or imbalance (Hasegawa *et al.*, 1986).

Tolerance mechanisms used by plants to adapt to salinity are varied. Salt avoidance mechanisms may involve exclusion at the root by selective uptake or ion exchange at the root cortex (Jeschke, 1984), reabsorption by xylem parenchyma cells and transport out of the root (Yeo *et al.*, 1977), redistribution to senescing leaves or other plant parts (Yeo and Flowers, 1984), and in halophytic plants, secretion or sequestration of ions into salt glands or bladders (Flowers *et al.*, 1977). To avoid osmotic imbalance, halophytic plants accumulate ions when grown under high salinity. However, enzymes from halophytes have been found to be as sensitive to salinity as those from glycophytes, and are not compatible with the levels of NaCl found in saps expressed from halophyte leaves (Flowers *et al.*, 1977).

It is generally accepted that salinity tolerance at the cellular level involves tight control of the ion levels of the cytoplasm, coupled with compartmentation of the excess "saline ions" required for osmotic adjustment within the vacuoles. The concentration of ions in the cytoplasm is held in the range 100-200 *mM* (200-400 mOsmol kg^{-1} osmotic pressure), with a strong preference for K^+ over Na^+. When the tissue concentration of NaCl exceeds about 200 *mM*, ion compartmentation becomes necessary to avoid enzyme deactivation and subsequent cell death. Under these conditions, the maintenance of osmotic equilibrium across the tonoplast requires the accumulation in the cytoplasm of nontoxic "compatible solutes", above a basal cytoplasmic osmotic pressure of 300-400 mOsmol kg^{-1} (Gorham *et al.*, 1985b; Wyn Jones, 1981).

Though there are a number of possible compatible solutes, the two most commonly found to accumulate in certain salt tolerant grasses tested are glycinebetaine and proline. Glycinebetaine has also been found to accumulate in dicotyledonous halophytes of the families Chenopodiaceae, Asteraceae, and others (Wyn Jones, 1981; Wyn Jones, 1984).

Little is known about the mechanisms of salinity tolerance of C_4 turfgrasses. Reported responses to salinity include differences in relative shoot growth reductions with increasing salinity in bermudagrass (Youngner and Lunt, 1967; Ramakrishnan and Nagpal, 1973; Dudeck *et al.*, 1983) and seashore paspalum cultivars (Dudeck and Peacock, 1985). Osmotic adjustment under saline stress occured in seashore paspalum (Peacock and Dudeck, 1985). Shoot Na^+ concentrations in bermudagrass and seashore paspalum increased when grown under saline

conditions, while K^+ concentrations declined (Dudeck *et al.*, 1983; Dudeck and Peacock, 1985; Leonard, 1983).

Knowledge of the relative salt tolerance of turfgrass species and their strategies of salt tolerance could allow better management decisions to be made, and improve the efficiency of breeding programs. This study was conducted to compare the relative salt tolerance, growth, and physiological responses of 6 C_4 turfgrasses to salinity in attempt to elucidate mechanisms of salinity tolerance.

MATERIALS AND METHODS

The experiment, which included 6 C_4 turfgrasses (Table 5), was conducted in a glasshouse using a solution culture system. Eight uniform sprigs were planted into each plastic pot filled with coarse silica sand. Pots were 9 cm in diameter by 6 cm deep with coarse screen bottoms which allowed roots to grow into the nutrient solution. Pots were suspended by white, 2 cm thick plywood sheets over tubs containing 12 L of a constantly aerified, modified Hoagland no. 2 solution (Hoagland and Arnon, 1950) in deionized water, in which 2 mg Fe L^{-1} was supplied as Fe-EDDHA chelate (Ciba-Geigy Sequestrene 138). Grasses were clipped every 10 days at 2.5 cm cutting height throughout the experiment, and were allowed to become fully established with a dense turf before treatments were begun. To avoid salinity shock, salinity levels were gradually increased by 50 *mM* NaCl (2.9 g NaCl L^{-1}) every day until final treatment levels of 1, 100, 200, 300, and 400 *mM* NaCl (0.054, 5.8, 11.7, 17.5, and 23.4 g L^{-1} NaCl) were reached. The control

Table 5. Turfgrasses evaluated for salinity tolerance.

Common name	Classification
Tifway bermudagrass	*Cynodon dactylon X C. transvaalensis* Burtt-Davey
Centipedegrass	*Eremochloa ophuriodes* (Munro) Hack.
Seashore paspalum (Hawaii selection)	*Paspalum vaginatum* Swartz
St. Augustinegrass (Hawaii selection)	*Stenotaphrum secondatum* Walt.
Japanese lawngrass	*Zoysia japonica* Steud
Manilagrass	*Zoysia matrella* (L.) Merr.

solution actually contained 1 *mM* NaCl (0.054 g L^{-1}), as better growth was observed when some NaCl was added to the nutrient solution in previous experiments. Thereafter, solutions were kept at a constant volume with deionized water, and nutrient solutions changed weekly, to avoid any change in salinity levels.

A clipping height of 2.5 cm was used throughout the experiment to stimulate turf-like growth. Shoots and roots (roots clipped at base of pot screens) were clipped 5 days after the highest salinity level was reached (400 *mM*), and discarded, allowing the plants to become equilibrated to the treatment salinities before clippings were taken for analysis. This clipping represented the initiation of the experiment. Thereafter, shoots were harvested at 10 day intervals for a total of 3 harvests. Immediately prior to clipping, shoots were thoroughly rinsed for 20 seconds in deionized water, then allowed to dry. Clipped shoots were immediately put into tared, 120 ml glass bottles with air-tight lids for fresh weight determination. During the final shoot harvest, roots growing through the screen were clipped, rinsed in deionized water for 20 seconds, and blotted dry.

Quality of the turf was evaluated twice for color and live shoot density 6 weeks folowing the initiation of the experiment, and the results averaged. Pots were rated on a 1 to 9 scale for color and live shoot density (1=completely brown turf-no live shoots; 9=completely green turf-no dead shoots).

For determination of shoot ion secretion through salt glands, a small amount of unrinsed shoots of grasses grown in 200 *mM* NaCl were clipped immediately prior to the final harvest, and placed in small,

tared plastic vials. The dry weights of these shoots were included in final harvest dry weights. Ion secretion was determined as the difference in ion contents between unrinsed and rinsed shoots grown at 200 *mM* NaCl.

Shoots and roots were dried in a forced-air dryer at 70°C for 48 hours for dry weight determination, then ground in a Wiley mill with a 20-mesh screen and placed in air-tight containers. Prior to ion analysis they were redried at 70°C, and 450 mg samples were ashed for 7 hours at 450°C .

Ash was dissolved in 1 *M* HNO_3 for 5 hours, then diluted with deionized water and allowed to sit overnight, then shaken before aliquots were taken for analysis. Sodium and K^+ were determined by flame emission spectrophotometry, and Ca^{2+} and Mg^{2+} by atomic adsorption spectrophotometry. Chloride was determined with an Orion Cl^- ion activity electrode.

Leaves for sap osmolality determination were placed in 1.5 ml plastic microcentrifuge tubes with air-tight covers and immediately frozen in dry ice. Two subsamples were taken per pot. Thawed tubes were flattened in a hydraulic press to release the leaf sap. Sap osmolality was measured with a Wescor model 5100C vapor pressure osmometer.

For proline determination, clipped leaves were placed in small air-tight plastic vials and immediately frozen in dry ice. The leaves were subsequently homogenized in 3% sulfosalicylic acid solution and assayed spectrophotometrically by an acid-ninhydrin method (Bates *et al.*, 1973).

The betaines (glycinebetaine and trigonelline) were determined by high performance liquid chromatography (Gorham, 1984). Dry tissue (0.1 g) was refluxed in methanol at 70°C for 1 hour, filtered, and reduced to dryness under nitrogen. The dried extract was partitioned by shaking in equal amounts of water and chloroform (3 mls each) for 5 minutes, then centrifuged, and the aqueous layer removed. Ion exchange resins were added to the aqueous extract in a ratio of 2:1 anion to cation exchanger (about 0.2 g total weight) which was sufficient to remove all inorganic ions, amino acids, organic acids, and zwitterions other than betaines (Hitz and Hanson, 1980). Dowex 1X2-100, a strong anion exchange resin, was regenerated in the OH^- form before use, and Amberlite IRC-50, a weak cation exchange resin, was used in the H^+ form. After shaking for 5 minutes, the resins were removed by centrifugation, and the deionized extract used directly for HPLC.

The HPLC system consisted of a Perkin-Elmer 410 HPLC pump with a 20μL injection loop, and a LC-95 variable wavelength UV detector. Separations were performed on a 250X5 mm I.D. stainless-steel column packed with Partisil 10-SCX and fitted with a direct-connect guard column packed with the same material. The buffered mobile phase consisted of 50 *mM* KH_2PO_4 plus 5% methanol, pH 4.6, and was millipore filtered and kept saturated with helium. Recovery of glycinebetaine averaged 88% using these methods in recovery trials (Appendix D).

All data were analyzed by regression using the "testing for heterogeneity of linear and quadratic effects" approach of Chapter 3. The overall goodness of fit of reduced models is described by both the model standard error and model R^2. Total mortality occured in both

centipedegrass and Japanese lawngrass at intermediate to higher salinities. Regressions were not attempted when there was incomplete data. Rather, means with accompanying standard errors are presented for these grasses. No root ion data is presented for centipedegrass, as there were insufficient roots present for analysis. Correlations between variables among individual grasses were compared using Pearson product-moment coefficients. In all figures, labels for the grasses have been abbreviated to: Ber. (Tifway bermudagrass), Cent. (centipedegrass), Japn. (Japanese lawngrass), Manl. (manilagrass), Pasp. (seashore paspalum), and Saug. (St. Augustinegrass).

RESULTS

Shoot growth, expressed as dry wt./week, varied greatly among grasses with increasing salinity (Fig. 15). St. Augustinegrass and seashore paspalum had much higher shoot growth rates across salinity than the other grasses. St. Augustinegrass shoot growth was stimulated under intermediate salinities. Manilagrass and Tifway bermudagrass had intermediate growth rates, but bermudagrass growth rates dropped off at high salinity. Centipedegrass and Japanese lawngrass shoot growth dropped off rapidly, and total shoot mortality occured at approximately 200 *mM* NaCl in centipedegrass.

Salinity tolerance is often expressed as relative reduction (percentage of control) in shoot growth rather than as total shoot growth reduction, with increasing salinity (Fig. 16). Using this approach the superior salinity tolerance of St. Augustinegrass, seashore

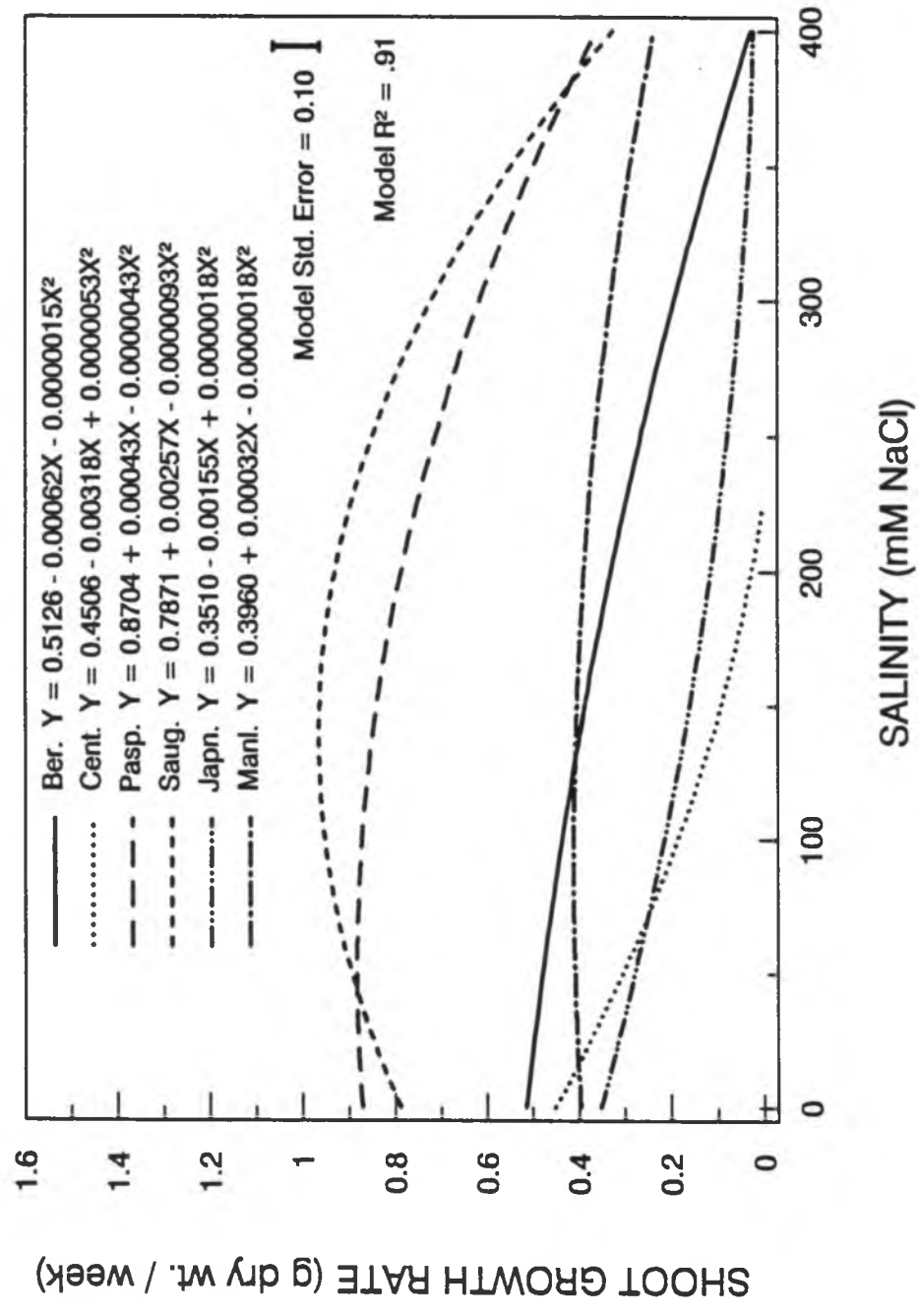


Figure 15. Shoot growth rates (g dry wt. week^{-1} pot^{-1}) as influenced by NaCl concentration.

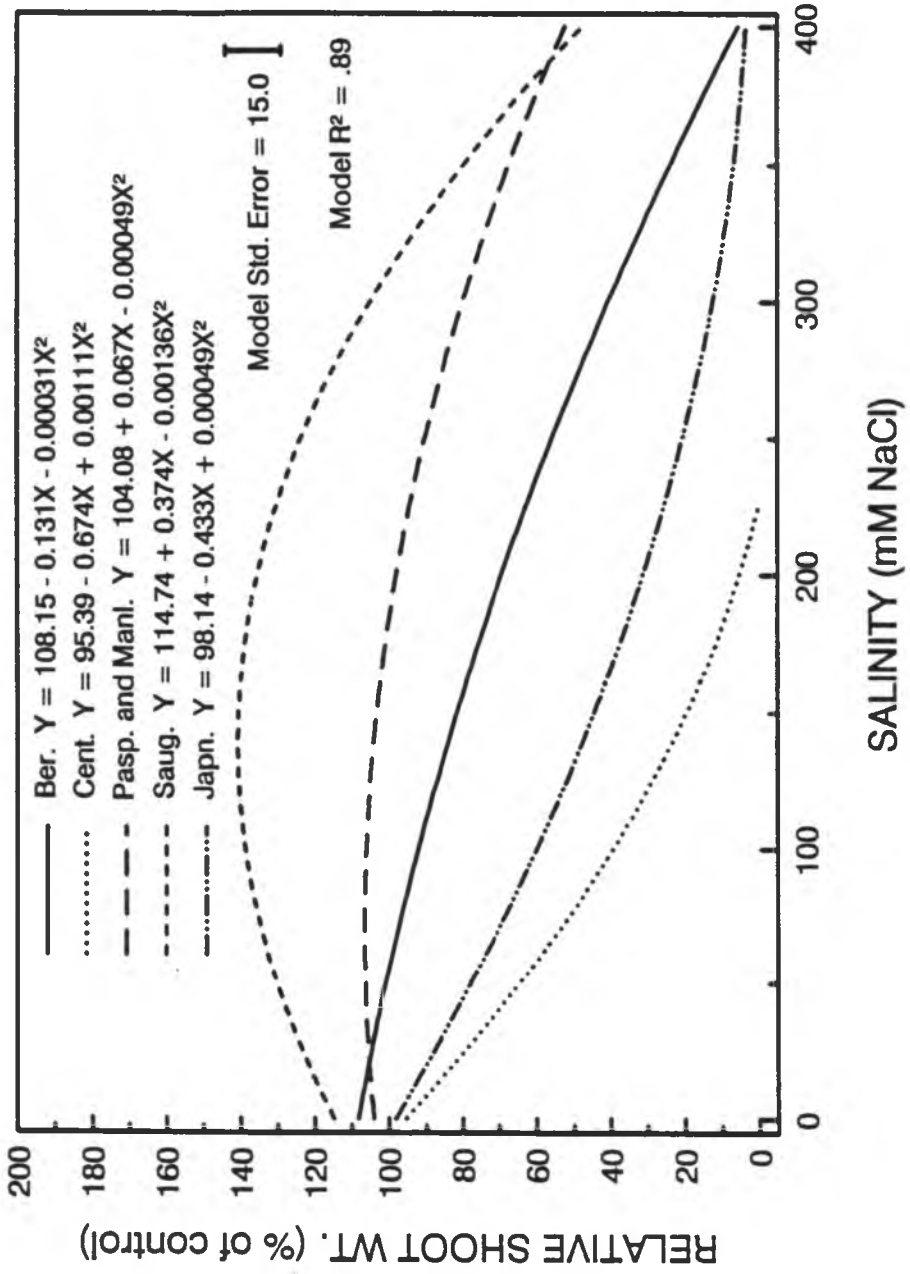


Figure 16. Relative shoot growth rates, expressed as % of control, as influenced by NaCl concentration.

paspalum, and manilagrass is evident. Relative shoot growth was reduced by only 50% at 400 *mM* NaCl over a period of 30 days in these three grasses. Relative shoot growth of St. Augustinegrass reached 140% under intermediate salinity due to growth stimulation, then dropped off. A predicted 50% relative shoot growth reduction occured at 270 *mM* NaCl in Tifway bermudagrass. Relative shoot growth dropped off rapidly in Japanese lawngrass and centipedegrass, with predicted 50% shoot growth reductions occurring at 130 and 80 *mM* NaCl, respectively.

Turfgrass quality, as indicated by visual ratings, followed the same trends as did relative shoot growth (Fig. 17). Seashore paspalum, St. Augustinegrass, and manilagrass maintained relatively dense, green turf under high salinity, seashore paspalum being slightly better than the rest. Bermudagrass quality dropped off much more rapidly, while Japanese lawngrass and centipedegrass quality plummeted. There were few live shoots remaining at the end of the experiment for Japanese lawngrass at 200 *mM* NaCl, and for centipedegrass, at 100 *mM*, respectively. However, seashore paspalum, St. Augustinegrass, and manilagrass continued to produce healthy shoots through the last harvest at 400 *mM* NaCl.

Shoot fresh wt./dry wt., a measure of tissue succulence, declined with increasing salinity in all grasses (Fig. 18). Shoot fresh/dry wt. of St. Augustinegrass and seashore paspalum remained about twice that of Tifway bermudagrass and the two zoysiagrasses (manilagrass and Japanese lawngrass) across all salinities. Centipedegrass had intermediate shoot fresh/dry wts. which declined rapidly at 100 to 200 *mM* salinity. Both

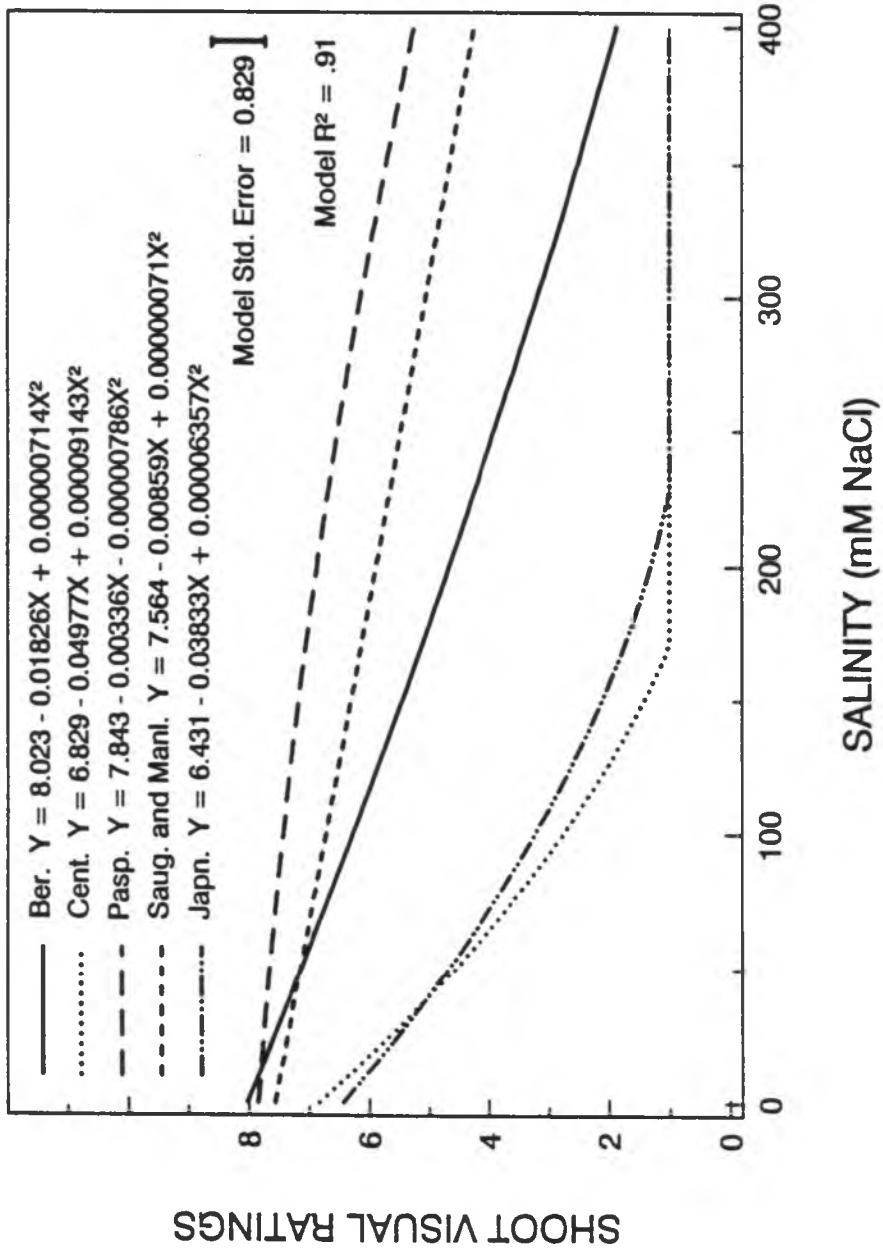


Figure 17. Shoot visual quality ratings as influenced by NaCl concentration. Shoots rated on a scale of 1-9 (1=completely brown turf-no live shoots; 9=completely green turf-no dead shoots).

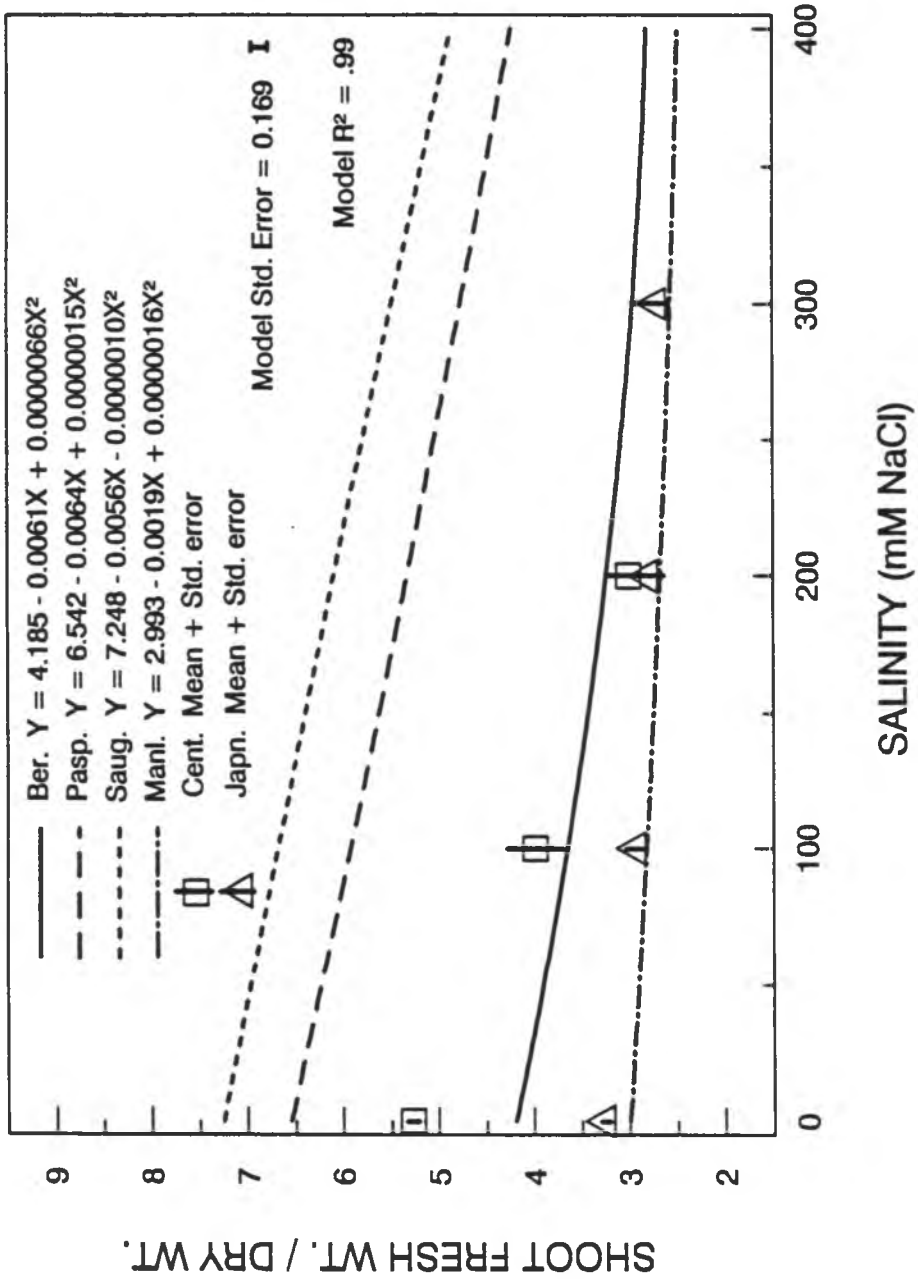


Figure 18. Shoot fresh weight/dry weight ratios as influenced by NaCl concentration.

zoysiagrasses had a low, but relatively constant shoot fresh/dry wt. ratio of approximately 3 across all salinities.

All grasses adjusted osmotically, maintaining their shoot sap osmolalities above that of the growing solution (Fig. 19). Sap osmolalities of Japanese lawngrass and bermudagrass reached very high values at high salinity (1160 and 1320 mOsmol kg^{-1}). This was due in part to the concentrating effect of low shoot fresh/dry wt. ratios. By comparison, the osmolality of sea water is about 1000 mOsmol kg^{-1}. Severe die-back occured in both grasses, with few live shoots remaining, at intermediate, or high salinity, respectively.

The saline ions Na^+ and Cl^- accumulated to high values in the shoots of St. Augustinegrass, reaching 1.7 and 0.8 mmol g^{-1} dry wt., respectively (Figs. 20 and 21). When expressed on a tissue water basis, the concentration of Na^+ exceeded 400 mmol L^{-1}. This is similar to concentrations reported in many dicotyledonous halophytes (Albert and Popp, 1977; Gorham *et al.*, 1980). Manilagrass, seashore paspalum, and Tifway bermudagrass restricted shoot uptake of Na^+ and Cl^- to a much higher degree. Japanese lawngrass accumulated medium shoot levels of Na^+ and Cl^-, while centipedegrass accumulated high Na^+ and very high Cl^- shoot levels when grown at only 100 *mM* salinity. When expressed on a tissue water basis, the concentrations of Na^+ and Cl^- in the shoots of these two grasses reached very high values (Figs. 22 and 23). This was partly due to the low shoot water contents of these grasses under salinity stress (Fig. 18).

Shoot K^+, Ca^{2+}, and Mg^{2+} decreased with increasing salinity in all grasses (Figs. 24-26). However, shoot K^+ was the predominant cation

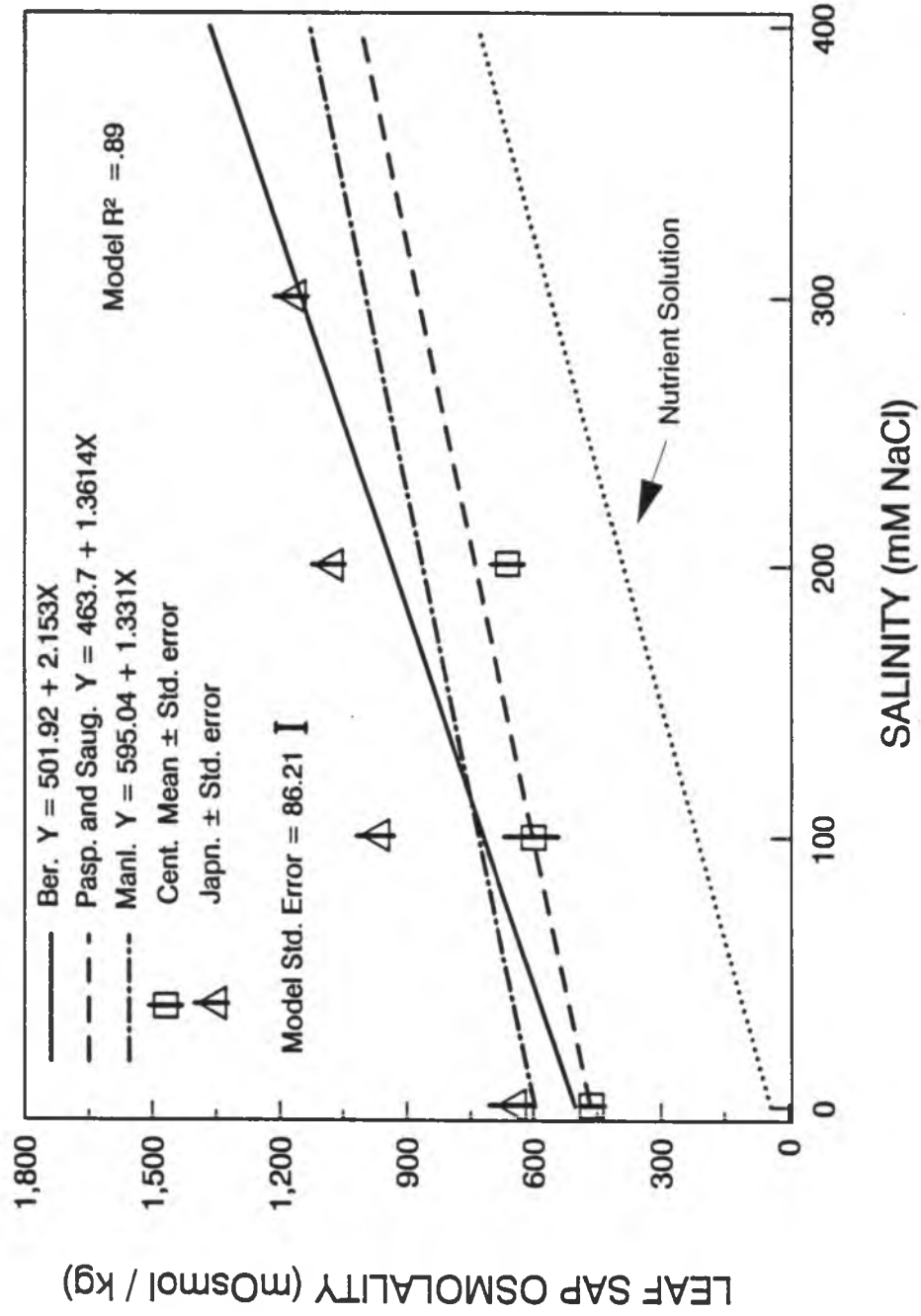


Figure 19. Leaf sap osmolalities as influenced by NaCl concentration.

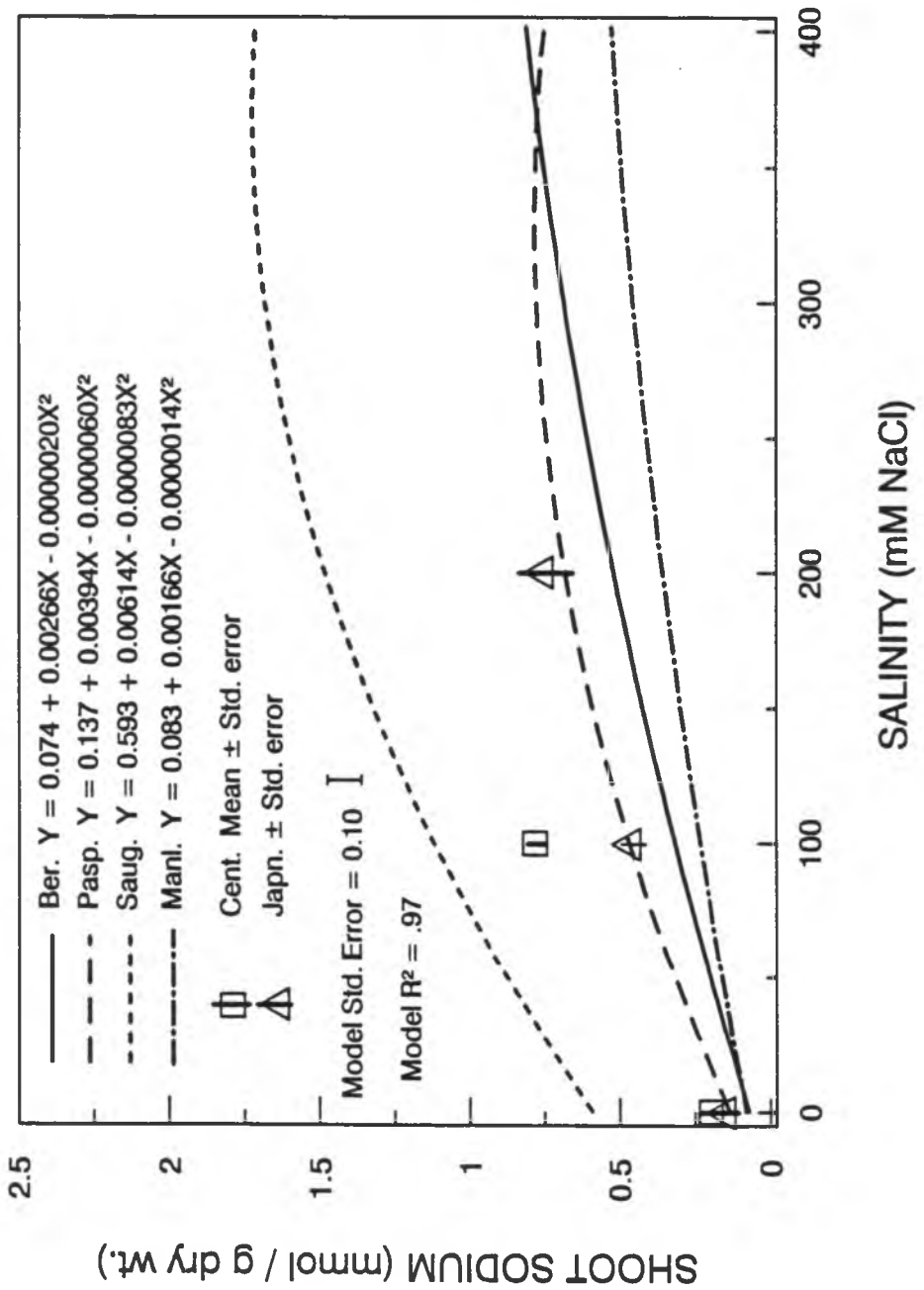


Figure 20. Shoot Na^+ concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.

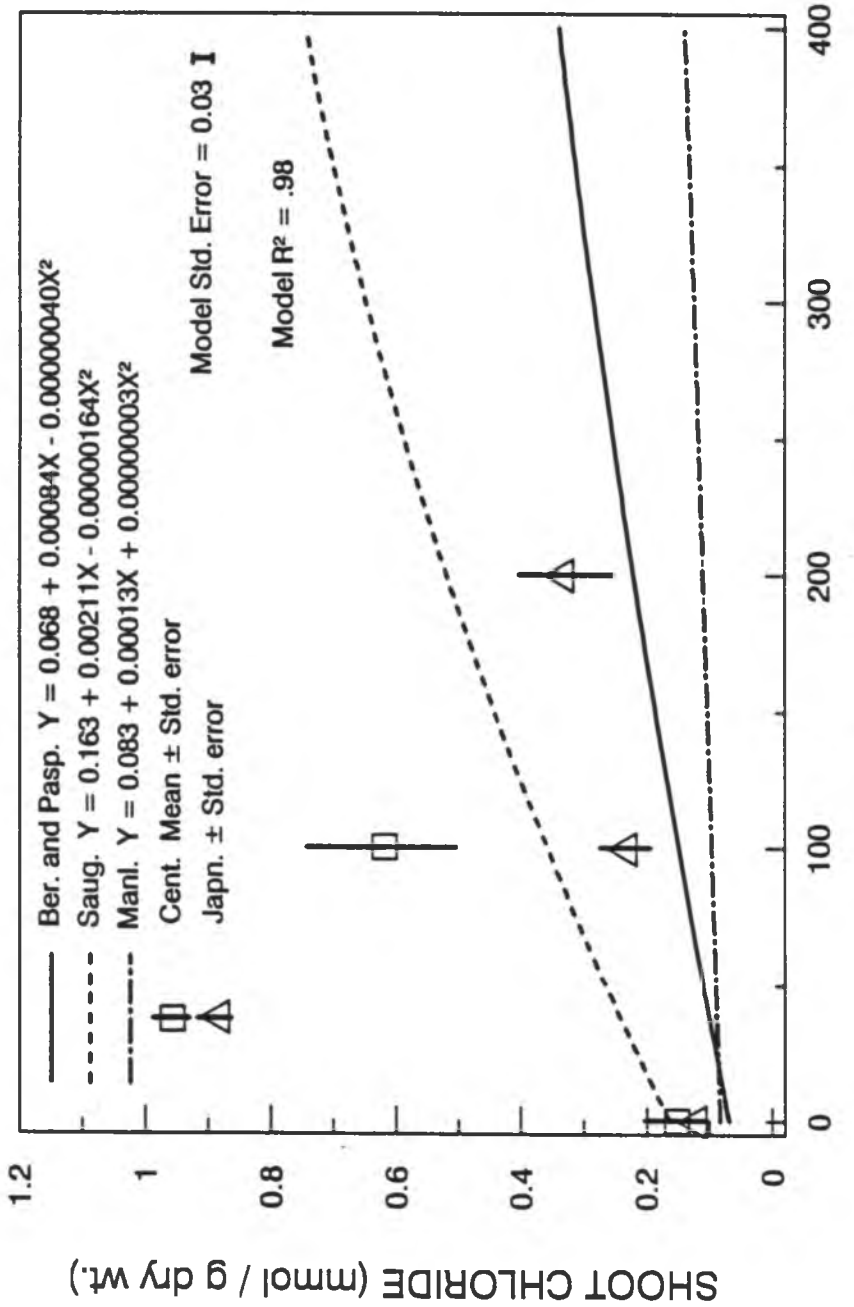


Figure 21. Shoot Cl^- concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.

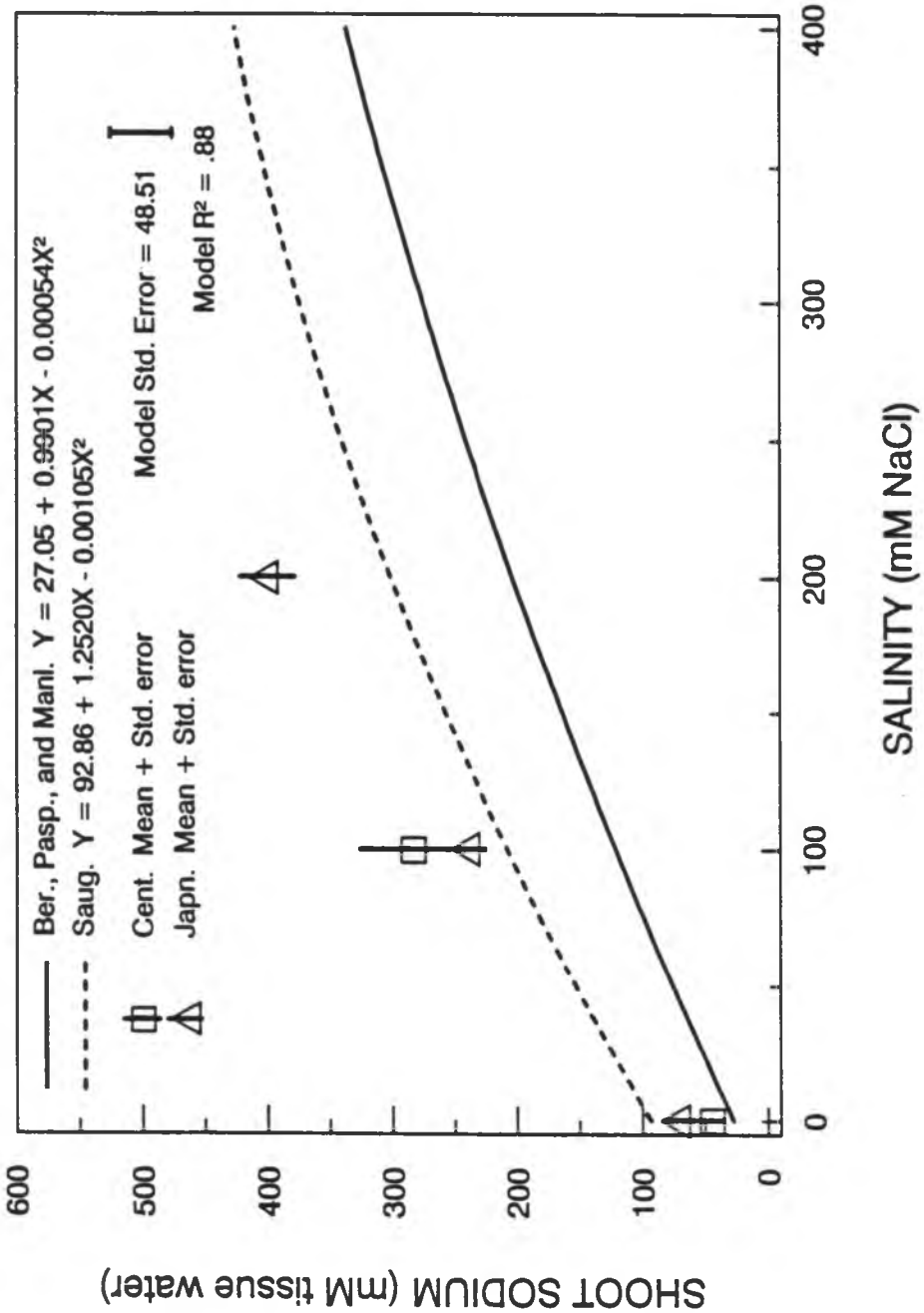


Figure 22. Shoot Na^+ concentrations, expressed on a tissue water basis, as influenced by NaCl concentration.

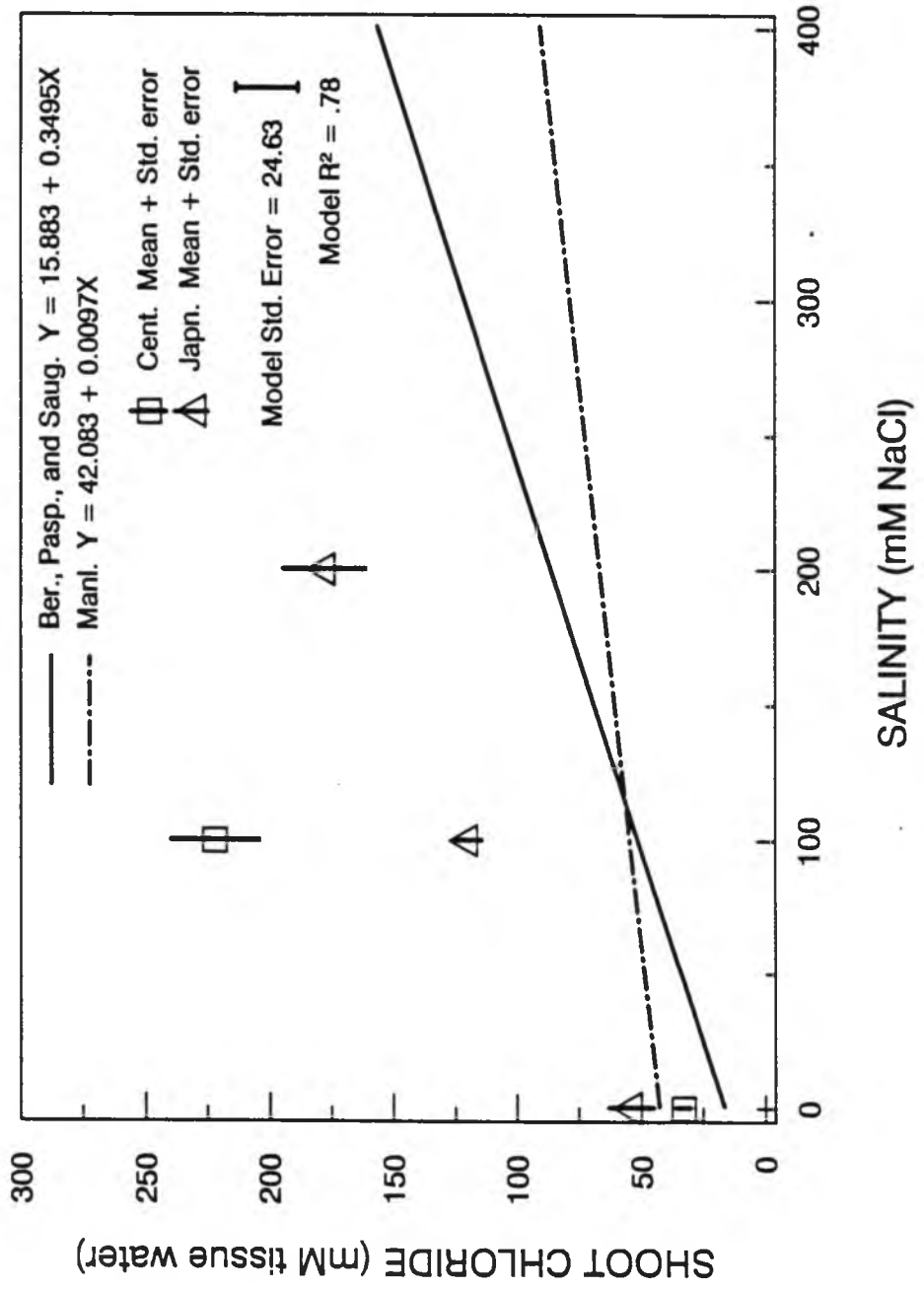


Figure 23. Shoot Cl^- concentrations, expressed on a tissue water basis, as influenced by NaCl concentration.

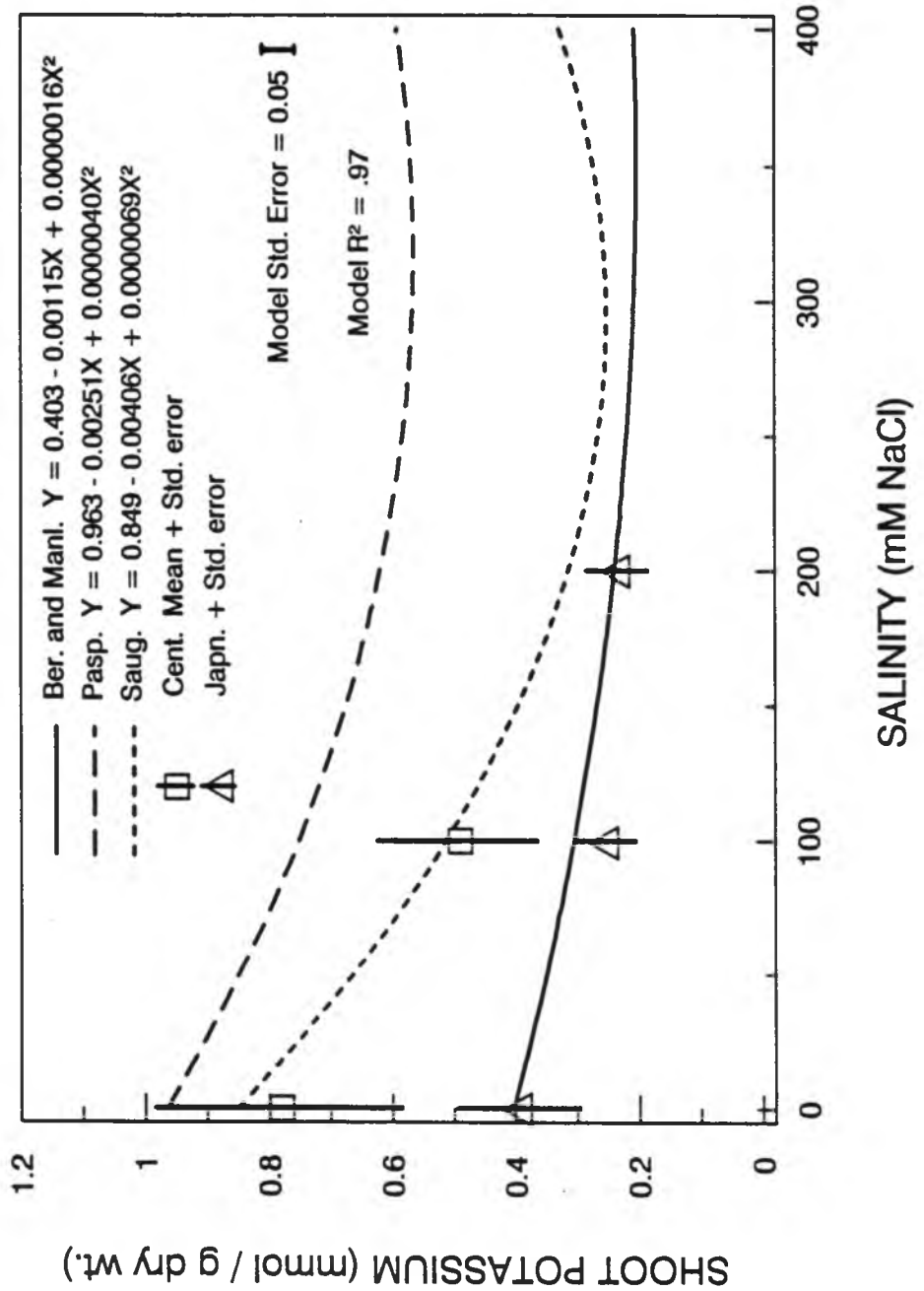


Figure 24. Shoot K^+ concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.

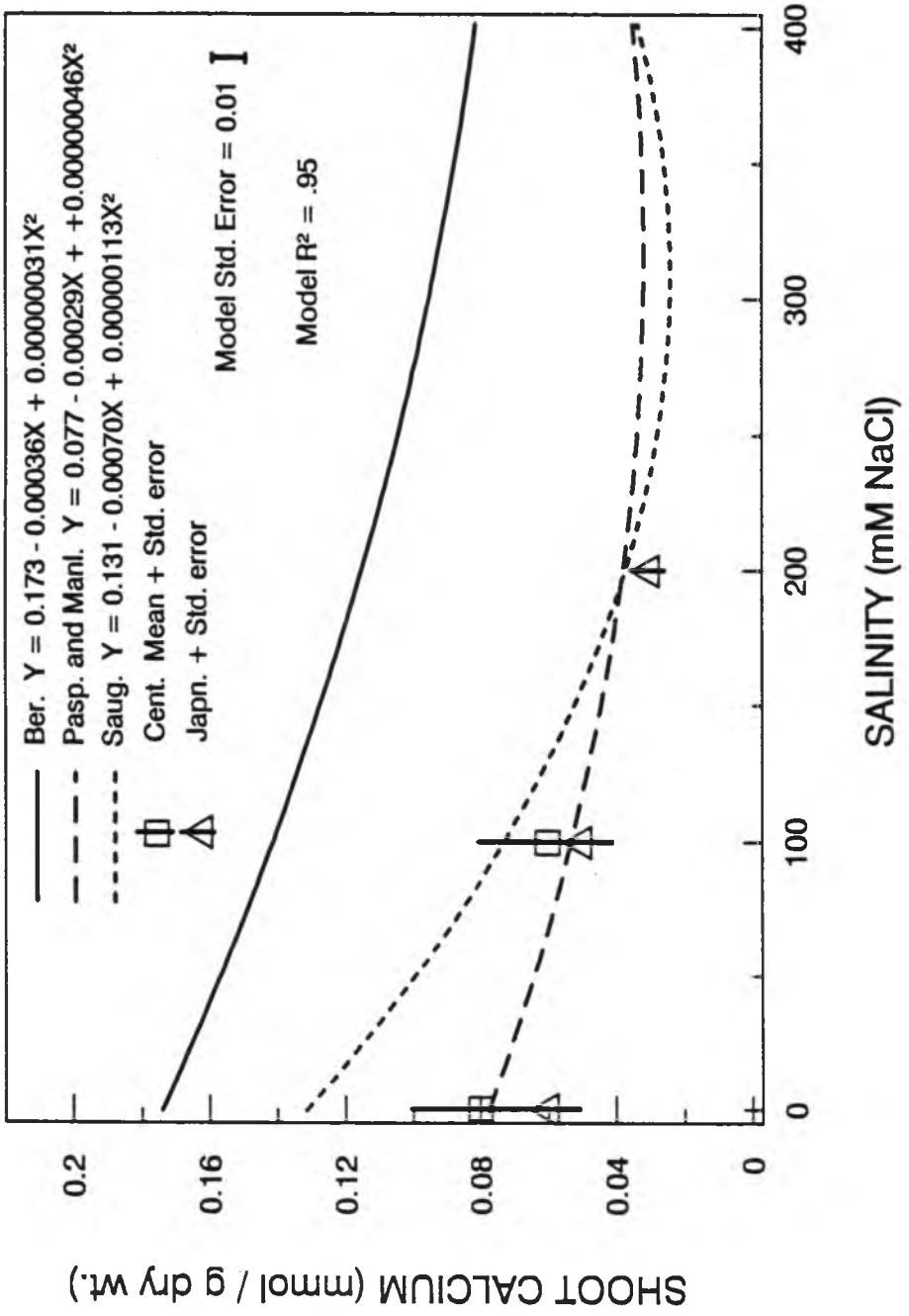


Figure 25. Shoot Ca^{2+} concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.

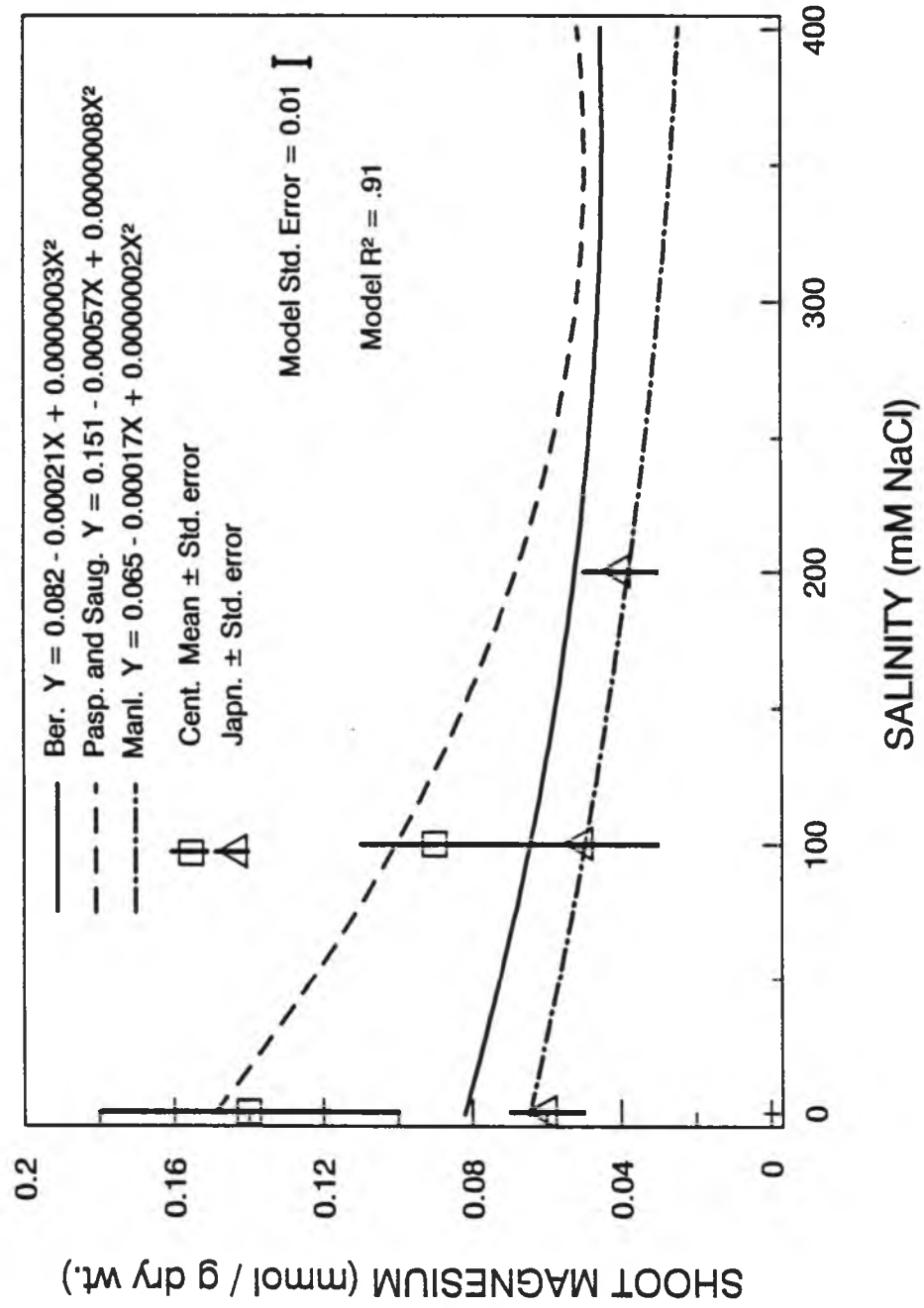


Figure 26. Shoot Mg^{2+} concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.

present in all grasses grown in the control treatments (1 *mM* NaCl). Seashore paspalum maintained a higher shoot K^+ content across all salinities than did the other grasses, which resulted in much higher shoot K^+/Na^+ ratios under salinity stress (0.73 at 400 *mM* NaCl), followed by manilagrass with a K^+/Na^+ ratio of 0.38 (Table 6). Tifway bermudagrass had relatively high shoot Ca^{2+} levels across all salinities.

Root Na^+ and Cl^- concentrations increased with increasing salinity in all grasses (Fig. 27 and 28). St. Augustinegrass had higher root Na^+ and Cl^- levels than did the other grasses, with the exception of seashore paspalum, which had equally high root Na^+ levels.

Shoot/root ratios of Na^+, and also of Cl^- were approximately 1 at higher salinities in St. Augustinegrass, Tifway bermudagrass, and Japanese lawngrass (Table 7) (data presented for Japanese lawngrass is at 200 *mM* NaCl) . However, for manilagrass and seashore paspalum, the shoot/root ratios of Na^+ at high salinity were approximately 0.5, and of Cl^- 0.2-0.3, respectively, indicating active exclusion of these ions from the shoots. Root K^+ decreased only slightly with increasing salinity in all grasses (Fig. 29). Root K^+ remained at high levels across salinity in seashore paspalum, as it did in the shoots. Root Ca^{2+} decreased with increasing salinity in all grasses (Fig. 30). Root Mg^{2+} remained relatively stable, except in seashore paspalum, where it increased substantially with increasing salinity (Fig. 31).

The presence of salt crystals on plants grown on saline media is indicative of active salt secretion by salt glands or bladders (Fahn, 1988). Salt crystals were observed on the leaves of Tifway

Table 6. Shoot K^+/Na^+ ratios as influenced by NaCl concentrations of 1 and 400 *mM*.

Grass	Shoot K^+/Na^+ ratios	
	1*mM*	400*mM*
Bermudagrass	4.84	0.26
Seashore paspalum	8.48	0.73
St. Augustinegrass	1.56	0.17
manilagrass	5.51	0.38
Japanese lawngrass[z]	2.58	0.40
nutrient solution	3.00	0.0075

[z]Data presented for Japanese lawngrass are from plants grown under 1 and 200 *mM* NaCl.

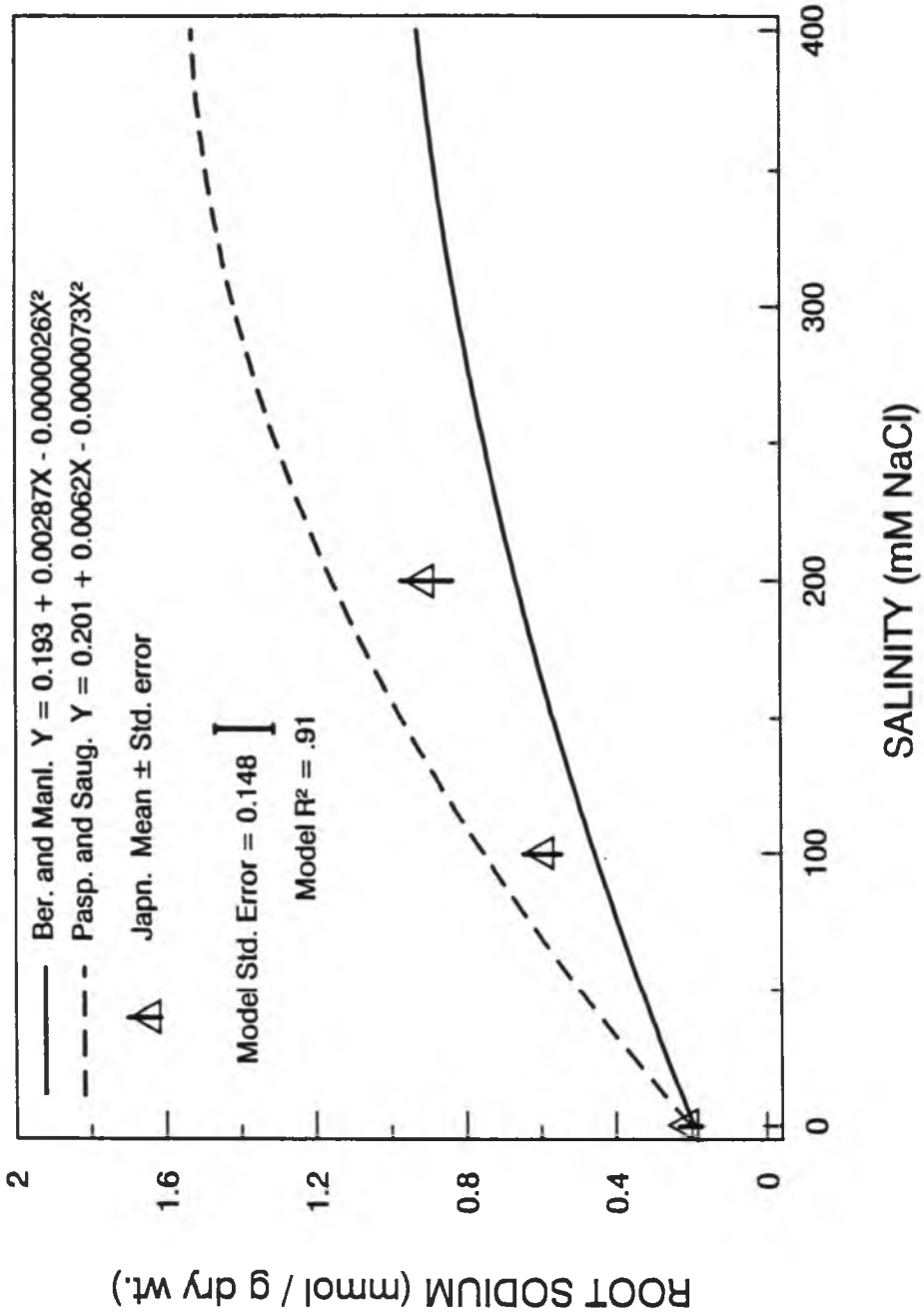


Figure 27. Root Na^+ concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.

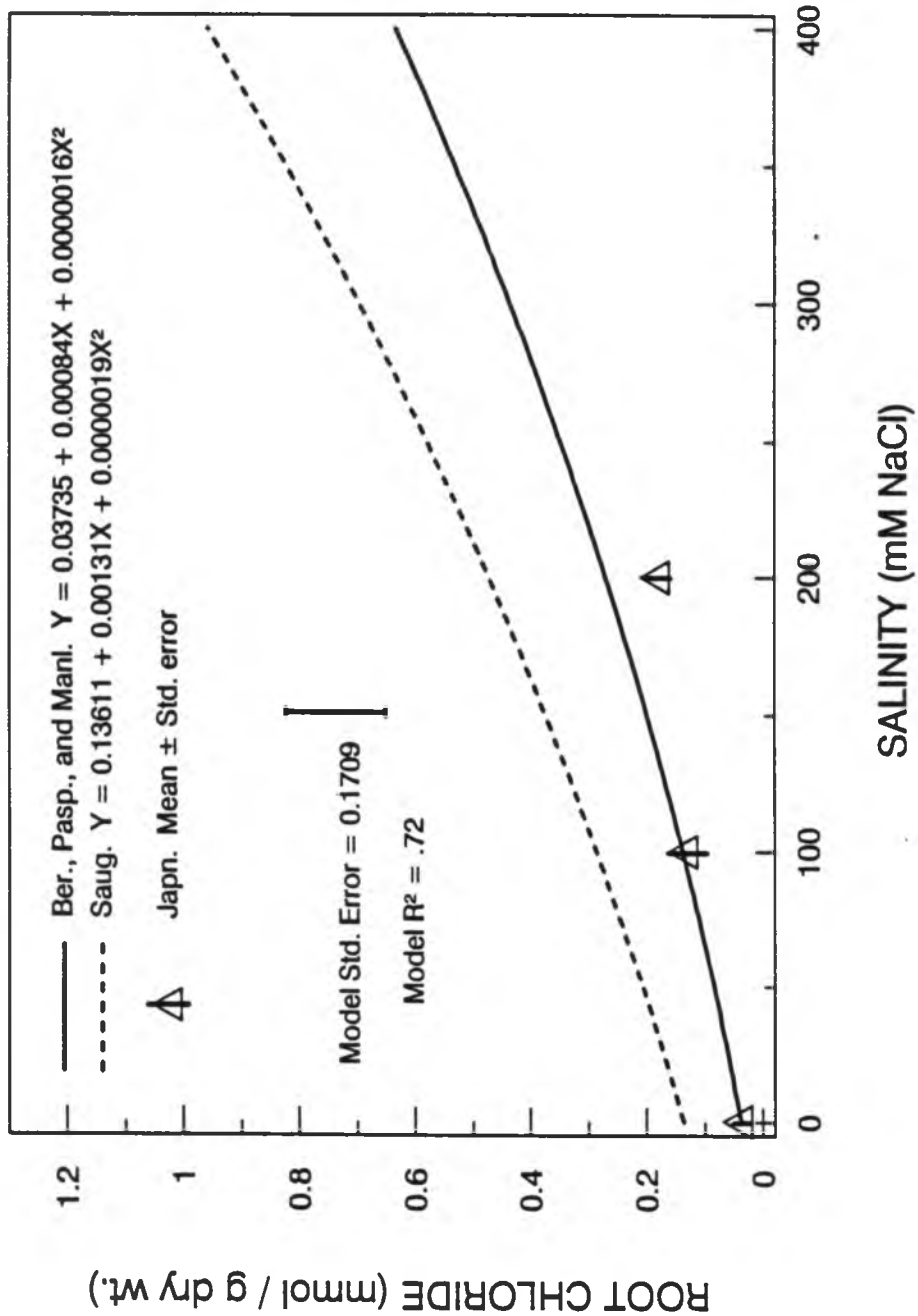


Figure 28. Root Cl^- concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.

Table 7. Shoot/root ratios of Na^+ and Cl^+ as influenced by 1 and 400 *mM* NaCl.

Grass	Shoot/root ratio Na^+		Cl^-	
	1mM	400mM	1mM	400mM
Bermudagrass	0.75	1.06	1.25	1.67
Seashore paspalum	0.50	0.55	2.51	0.34
St. Augustinegrass	2.58	1.08	1.14	0.88
manilagrass	0.28	0.46	2.13	0.19
Japanese lawngrass[z]	0.15	0.83	3.22	1.88

[z]Data presented for Japanese lawngrass are from plants grown under 1 and 200 *mM* NaCl.

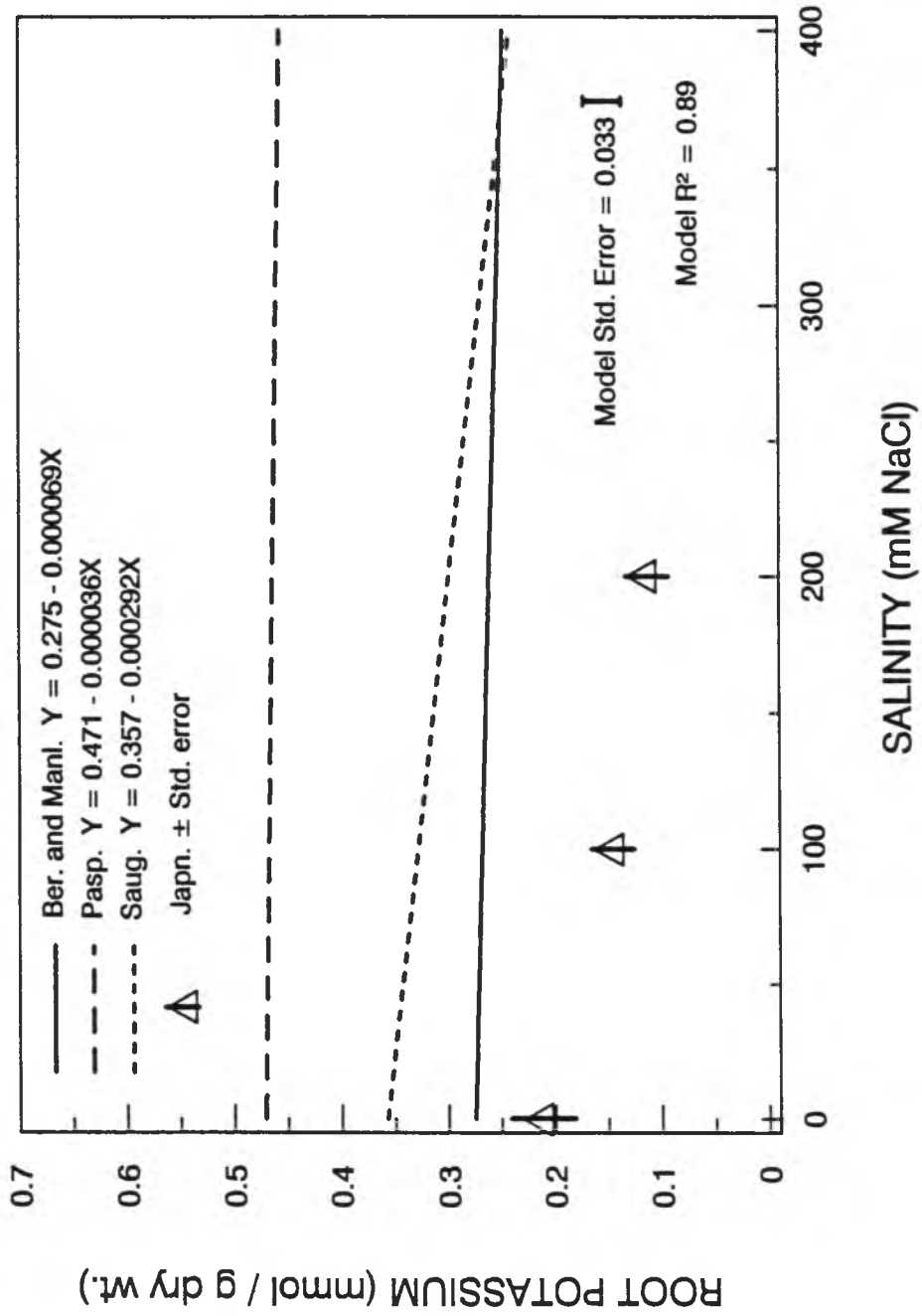


Figure 29. Root K^+ concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.

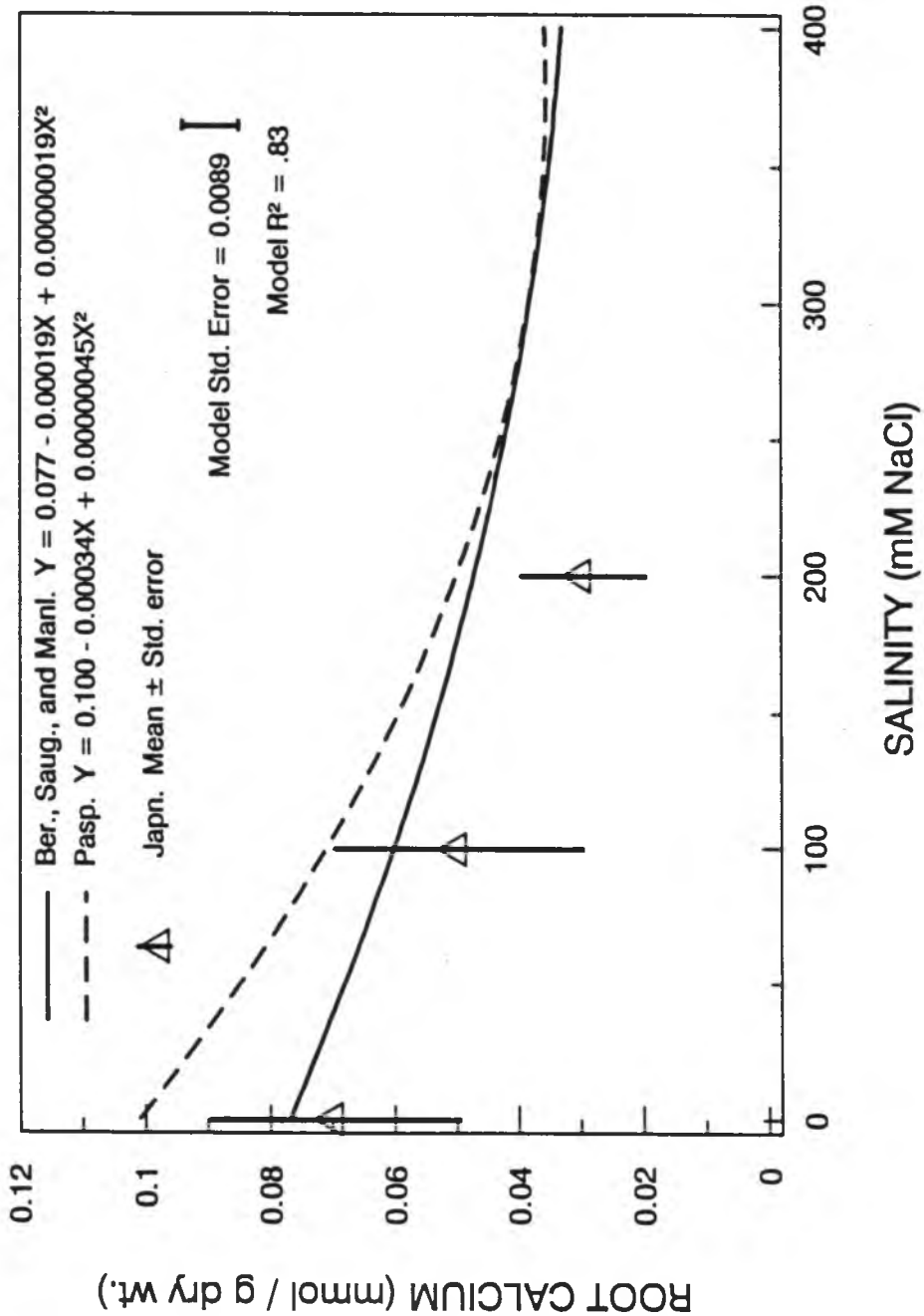


Figure 30. Root Ca^{2+} concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.

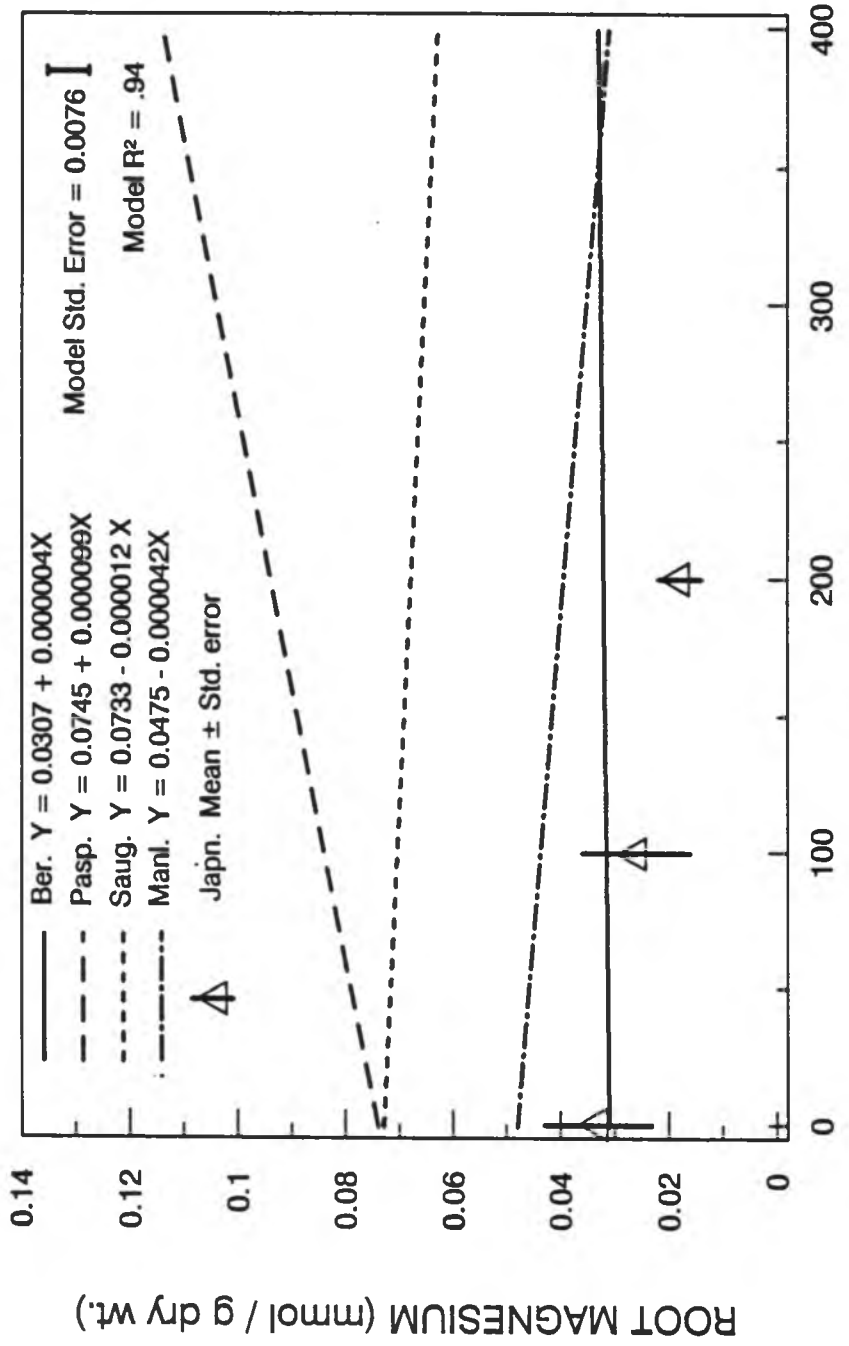


Figure 31. Root Mg^{2+} concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.

bermudagrass, manilagrass, and Japanese lawngrass in plants grown under intermediate to high salinity. Salt secretion is shown in Figure 32 as the difference in ion concentrations measured between unrinsed and rinsed leaves grown at 200 *mM* NaCl. Sodium and Cl^- secretion occured in all three grasses. A slight K^+ secretion is indicated for Tifway bermudagrass and manilagrass, but the trend is not significant for Japanese lawngrass. Manilagrass, Tifway bermudagrass, and Japanese lawngrass shoots secreted 0.72, 0.66, and 0.36 mmol g^{-1} dry wt. Na^+, and 0.42, 0.43, and 0.24 mmol g^{-1} dry wt. Cl^-, respectively, over a 10 day period. This was confirmed visually, as the leaves of manilagrass and Tifway bermudagrass had much denser deposits of salt than did the leaves of Japanese lawngrass.

Proline accumulated to a level of 200 μmol g^{-1} dry wt. in Tifway bermudagrass at 400 *mM* NaCl (Fig. 33), which is high when compared to a number of other halophytic plants (Briens and Larher, 1982), but not as high as levels found in *Puccinellia* (Poaceae) and *Triglochin* species. (Stewart and Lee, 1974). Proline has been previously reported to accumulate in common bermudagrass under both salinity and drought stress (Manetas *et al.*, 1986; Stewart and Lee, 1974). Proline levels in St. Augustinegrass, seashore paspalum, manilagrass, and Japanese lawngrass at 400 *mM* NaCl were modest, averaging 40 μmol g^{-1} dry wt.

Glycinebetaine accumulated most rapidly and reached highest levels in Tifway bermudagrass and seashore paspalum, reaching levels of approximately 200 μmol g^{-1} dry wt. at 400 *mM* NaCl (Fig. 34). This is equal to the level of proline found in Tifway bermudagrass at this salinity. In St. Augustinegrass, manilagrass, and Japanese lawngrass

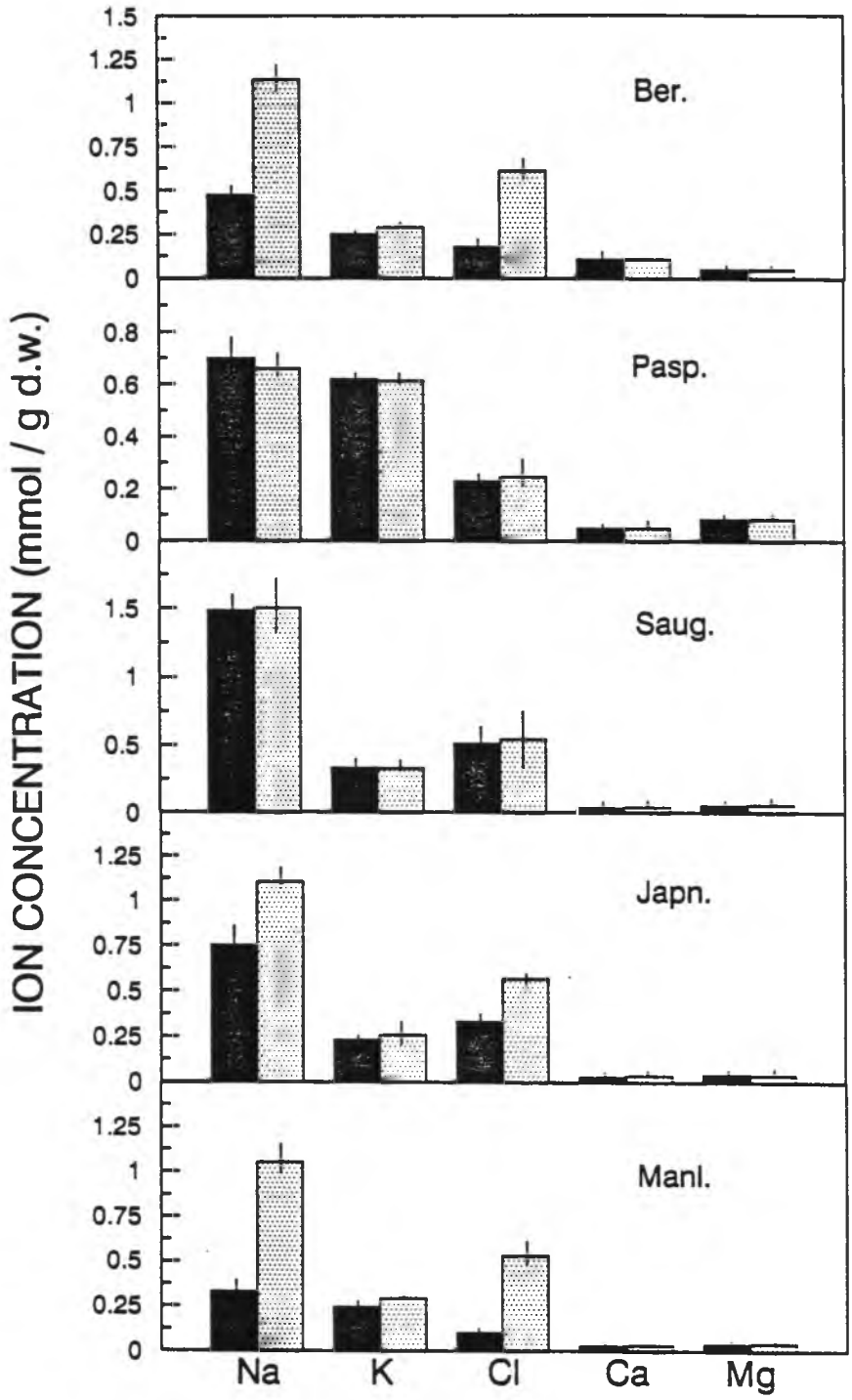


Figure 32. Ion concentrations in leaves of grasses grown in 200 *mM* NaCl. Light shade-unrinsed leaves, dark shade-rinsed leaves. Differences represent ion secretion for 1 week. Results are means ±s.e. of 3 plants.

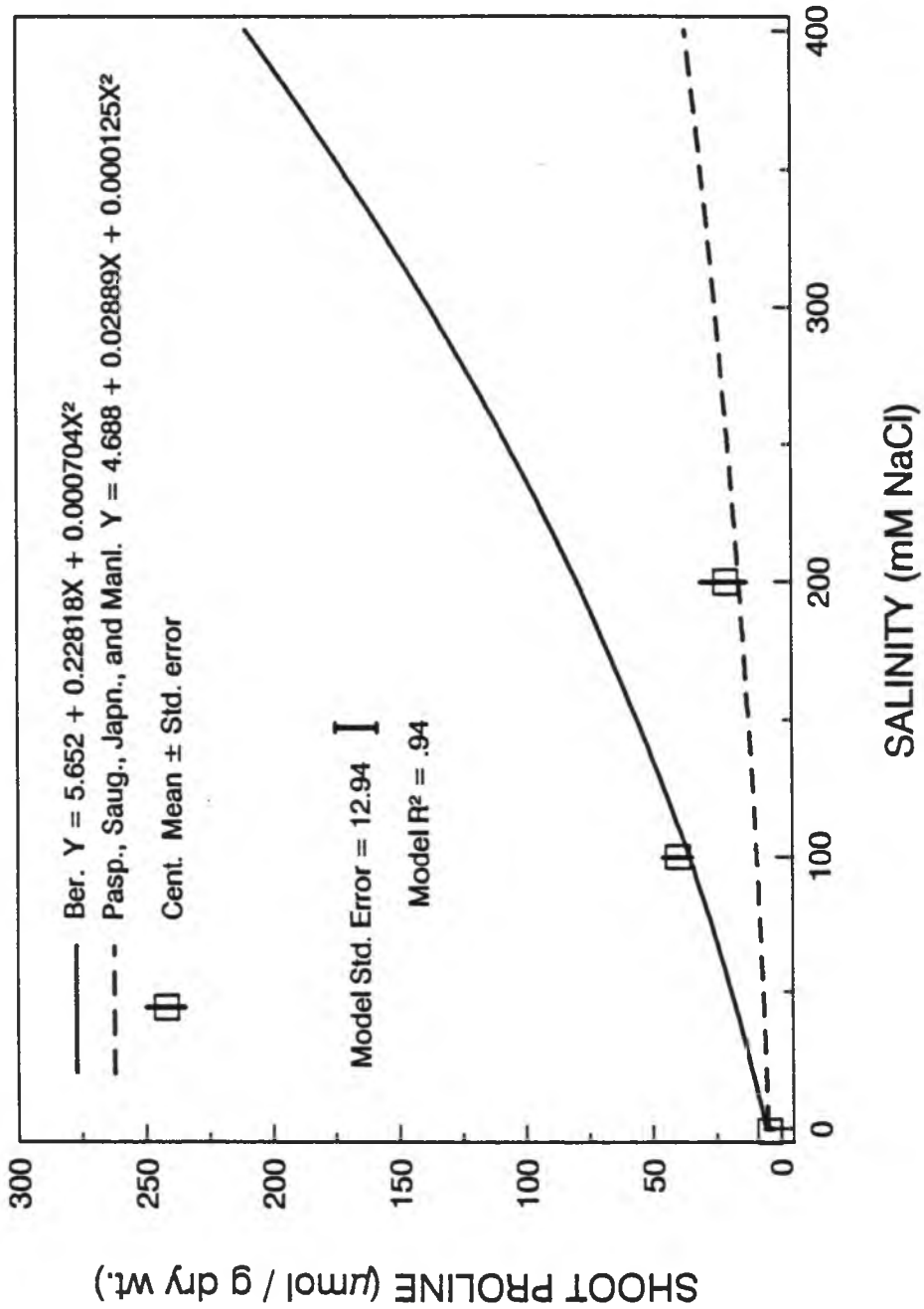


Figure 33. Shoot proline concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.

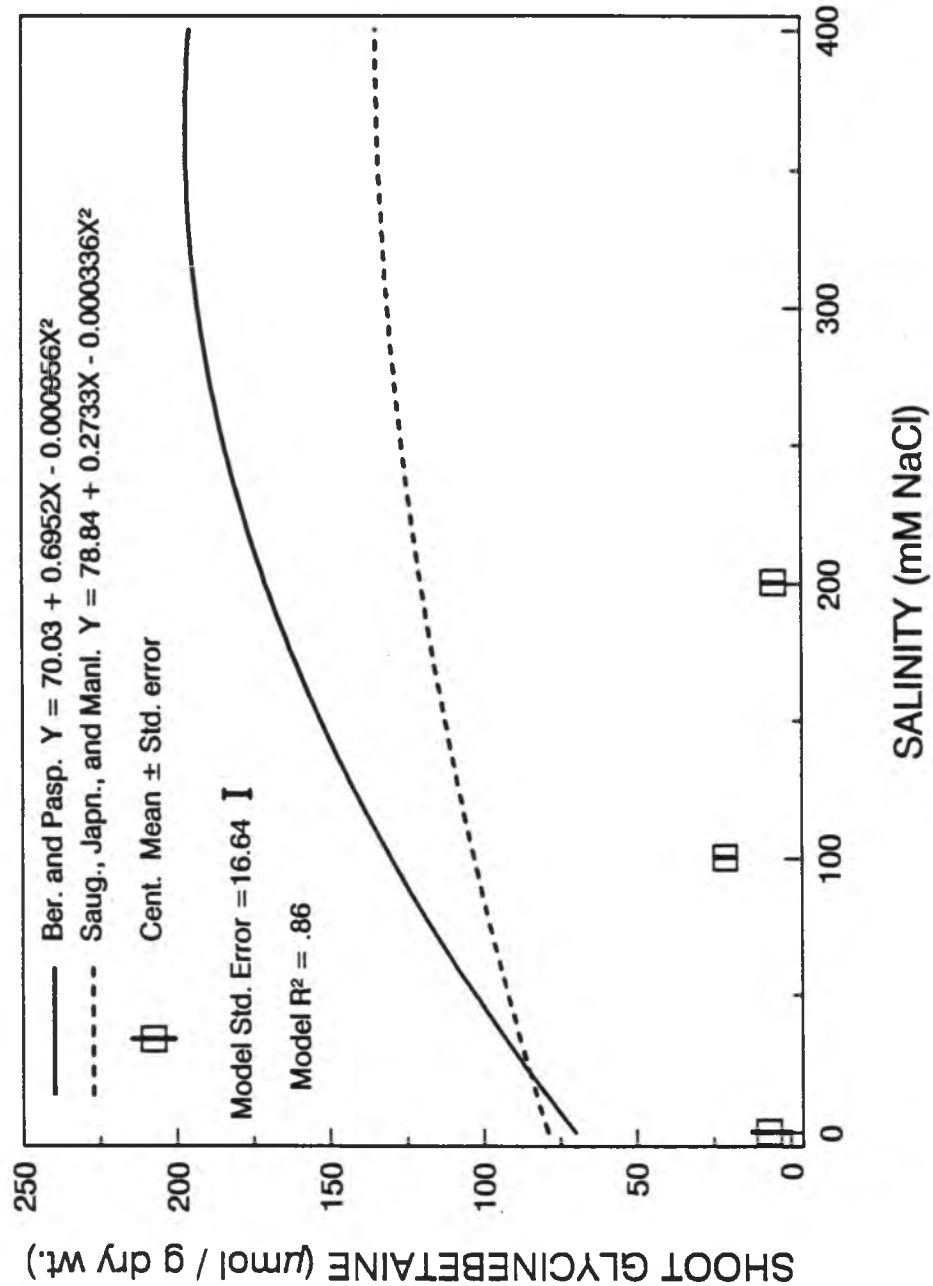


Figure 34. Shoot glycinebetaine concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.

accumulation of glycinebetaine was not as rapid, nor were levels as high at 400 *mM* NaCl. No significant accumulation of glycinebetaine occured in centipedegrass, which had very low shoot levels. Shoot glycinebetaine concentrations in Tifway bermudagrass and seashore paspalum were higher than those reported in barley, wheat, sorghum, and other glycophytic and mesophytic grasses which have been studied (Hitz and Hanson, 1980; Wyn Jones and Storey, 1978a; Grieve and Maas, 1984), but were similar to the levels found in the halophytic grasses *Spartina townsendii* and *Sporobolus* spp. (Wyn Jones and Storey, 1981), both of the subfamily Chloridoideae, of which bermudagrass is a member.

Trigonelline (nicotinic acid betaine) has been found to occur in a number of grasses in conjunction with glycinebetaine, albeit at lower levels. Trigonelline was found at very low levels in all grasses except centipedegrass (Fig. 35). St. Augustinegrass had higher trigonelline levels than did the other grasses, and also some increase in concentration with increasing salinity occured.

Pearson product-moment correlation coefficients were compared for the variables shoot Na^+, Cl^-, K^+, Ca^{2+}, proline, glycinebetaine, sap osmolality, and relative growth rate (Table 8). For all grasses, there were good positive correlations between sap osmolality and shoot Na^+ and Cl^- concentrations ($r > 0.85$), while sap osmolality, Na^+, and Cl^- were all highly negatively correlated with K^+ and Ca^{2+}. Shoot proline was positively correlated ($r > 0.85$) with sap osmolality, except in Japanese lawngrass. There were good positive correlations between glycinebetaine and sap osmolality in Tifway bermudagrass, seashore paspalum, manilagrass, and Japanese lawngrass.

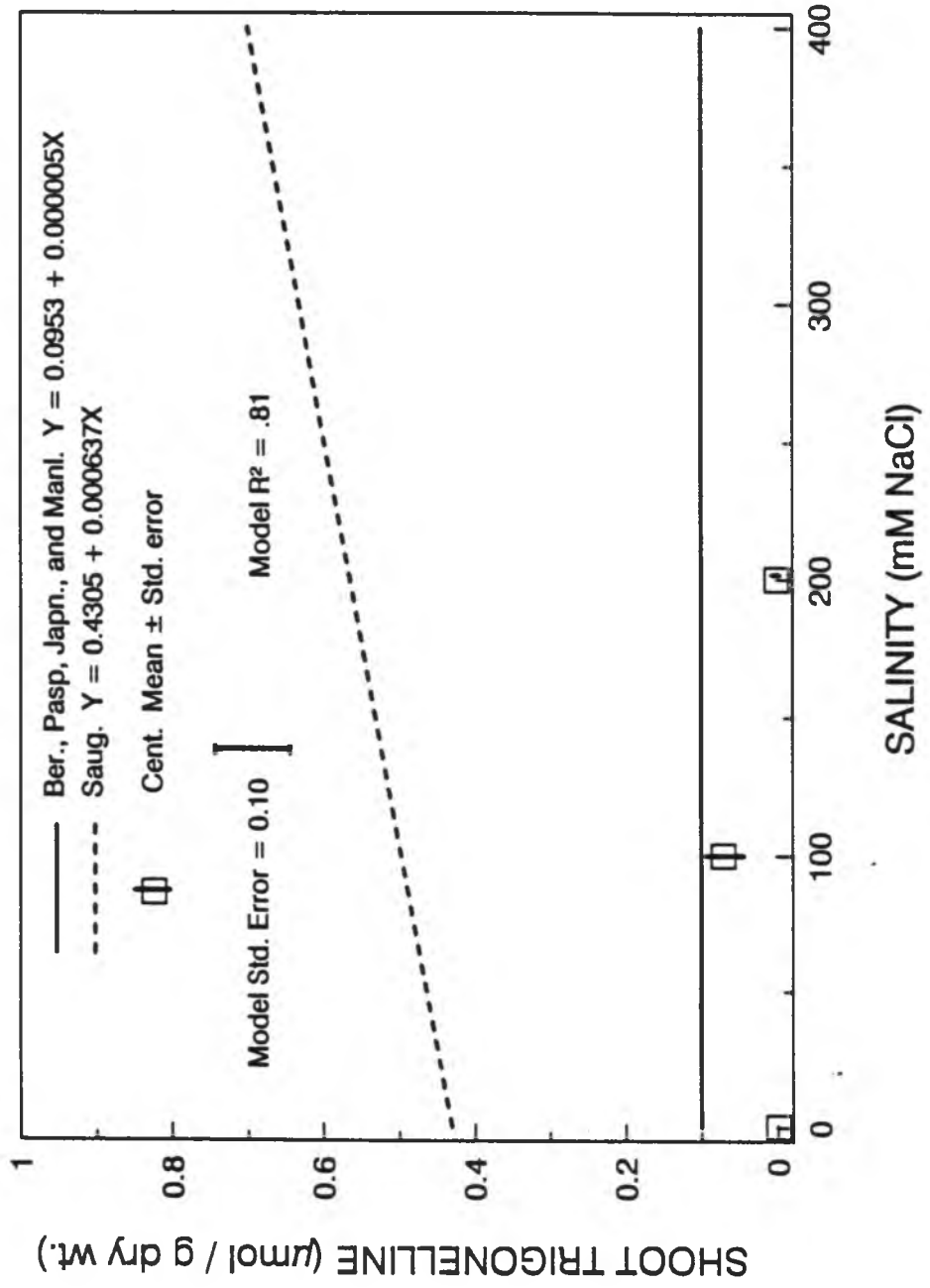


Figure 35. Shoot trigonelline concentrations, expressed on a dry weight basis, as influenced by NaCl concentration.

Table 8. Pearson correlation coefficients among selected variables.

Variable	Na	Cl	K	Ca	Osmol.	Prol.	Glybet.
Tifway bermudagrass							
Cl	0.99						
K	-0.87	-0.86					
Ca	-0.92	-0.92	0.92				
Osmolality	0.96	0.97	-0.87	-0.93			
Proline	0.91	0.94	-0.80	-0.88	0.94		
Glycinebet.	0.85	0.81	-0.93	-0.91	0.83	0.73	
Relative d.w.	-0.91	-0.92	0.73	0.85	-0.93	-0.93	-0.71
Centipedegrass							
Cl	0.98						
K	-0.99	-0.99					
Ca	-0.97	-0.93	0.94				
Osmolality	0.97	0.94	-0.92	-0.99			
Proline	0.99	0.99	-0.99	-0.96	0.90		
Glycinebet.	0.43	0.49	-0.54	-0.33	0.01	0.59	
Relative d.w.	-0.98	-0.99	0.99	0.93	-0.77	-0.90	-0.14
Seashore paspalum							
Cl	0.92						
K	-0.97	-0.91					
Ca	-0.94	-0.89	0.95				
Osmolality	0.89	0.93	-0.88	-0.84			
Proline	0.73	0.88	-0.71	-0.72	0.88		
Glycinebet.	0.92	0.91	-0.88	-0.85	0.91	0.82	
Relative d.w.	-0.54	-0.78	0.53	0.59	-0.78	-0.90	-0.74
St. Augustinegrass							
Cl	0.93						
K	-0.94	-0.86					
Ca	-0.97	-0.87	0.98				
Osmolality	0.92	0.94	-0.77	-0.82			
Proline	0.67	0.73	-0.59	-0.59	0.85		
Glycinebet.	0.71	0.57	-0.77	-0.76	0.57	0.50	
Relative d.w.	-0.26	-0.44	0.01	0.09	-0.54	-0.51	0.12
Japanese lawngrass							
Cl	0.95						
K	-0.92	-0.91					
Ca	-0.83	-0.87	0.66				
Osmolality	0.87	0.95	-0.93	-0.69			
Proline	0.04	0.02	-0.32	0.25	0.54		
Glycinebet.	0.96	0.94	-0.99	-0.72	0.87	0.87	
Relative d.w.	-0.86	-0.87	0.92	0.67	-0.90	-0.90	-0.60
manilagrass							
Cl	0.88						
K	-0.91	-0.82					
Ca	-0.86	-0.69	0.91				
Osmolality	0.91	0.89	-0.89	-0.78			
Proline	0.80	0.81	-0.80	-0.59	0.89		
Glycinebet.	0.74	0.59	-0.80	-0.74	0.77	0.55	
Relative d.w.	-0.51	-0.53	0.35	0.24	-0.74	-0.75	-0.35

DISCUSSION

Shoot growth rates were very high in St. Augustinegrass and seashore paspalum, and particularly under intermediate salinity in St. Augustinegrass, where a substantial stimulation of growth occured. Shoot growth exceeded 0.9 g dry wt./pot/week at 100 *mM* NaCl in St. Augustinegrass. When expressed on a fresh weight basis, the weekly clippings weighed over 6 g/pot, compared to 1.8 g/pot for Tifway bermudagrass at 100 *mM* NaCl.

These high growth rates were directly related to maintenance of shoot tissue succulence. Shoot fresh wts./dry wts. in St. Augustinegrass and seashore paspalum were fully double those of the other grasses across all salinities. A common response of plants, and particularly of grasses, to salinity is shoot dehydration due to a loss of cell turgor, resulting in reduced growth rates (Neumann *et al.*, 1988; Gorham *et al.*, 1980), as growth is intimately tied to the maintenance of cell turgor (Hsiao and Bradford, 1983). St. Augustinegrass and seashore paspalum are members of the subfamily Panicoideae (Gould and Shaw, 1983), whereas Tifway bermudagrass, manilagrass , and Japanese lawngrass, members of the subfamily Chloridoideae, had much lower shoot fresh wts./dry wts. and shoot growth rates across all salinities.

Determining the relative salinity tolerance among plants of divergent species is difficult, as there are no standard "markers" with which to compare plant vigor under stress. However, a comparison of the relative yield reduction, as a percent of control, with increasing salinity, and a comparison of the salinity level resulting in a 50%

relative yield reduction are often used (Maas and Hoffman, 1977). Using the 50% relative yield reduction as a criteria, St. Augustinegrass, seashore paspalum, and manilagrass were most tolerant, with 50% yield reductions occurring at about 400 *mM* NaCl, followed by Tifway bermudagrass at 270 *mM*, Japanese lawngrass at 130 mm, and centipedegrass at only 80 *mM* NaCl.

Although relative yield is a good measure of vigor, it is not a critical factor in turfgrass management. Indeed, a reduced growth rate may result in lower maintenance costs. Turfgrass quality, as measured by live shoot density, color, and texture, is more important from a turfgrass management perspective. Turfgrass quality rankings followed the same trends as did relative shoot reductions. Turf quality was best in seashore paspalum and St. Augustinegrass across all salinities, but was slightly better in seashore paspalum, mainly due to higher live shoot densities. Tifway bermudagrass was generally intermediate in quality, and maintained good leaf color at high salinity, though live shoot growth dropped off drastically. Leaf burn occured in both centipedegrass and Japanese lawngrass at 100 *mM* NaCl, and shoot dieback at low to intermediate salinities, respectively.

Osmotic adjustment occured in all grasses, although the difference between the osmolalities of the shoots and that of the nutrient solution decreased with increasing salinity. This has also been reported to occur in barley (Storey and Wyn Jones, 1978b). Osmotic adjustment was accomplished primarily by an increase in shoot Na^+ and Cl^- contents on a dry weight basis in all grasses. However, tissue dehydration, resulting in concentration of the cell sap, was also involved. The combination of

these two factors in osmotic adjustment has been reported to occur in other grasses (Storey and Wyn Jones, 1978b; Gorham *et al.*, 1984).

Salinity tolerance in halophytic grasses is often associated with exclusion of Na^+ salts from the shoots, but this often results in shoot dehydration and reduced growth (Gorham *et al.*, 1985b). Seashore paspalum, manilagrass, and Tifway bermudagrass maintained shoot Na^+ and Cl^- at low levels under high salinity. Shoot ion exclusion has been correlated with salinity tolerance among grasses of the same or closely related species (Hannon and Barber, 1972; Shannon, 1978; Gorham *et al.*, 1986). Seashore paspalum and manilagrass actively excluded Na^+ and Cl^- from the shoots, maintaining Na^+ and Cl^- shoot/root ratios at much less than 1, even when grown under 400 *mM* NaCl (Table 7). The other grasses had Na^+ and Cl^- shoot/root ratios of about 1 at 400 *mM* NaCl.

A high shoot selectivity for K^+ is involved in maintaining minimum basal K^+ shoot levels which are required in the cytoplasm for translation and other processes (Wyn Jones, 1984). Active discrimination for K^+ over Na^+ occurs at the root cortex, through selective K^+ uptake/Na^+ extrusion at the plasmalemma or K^+/Na^+ exchange at the tonoplast. Both processes restrict Na^+ from shoots, while releasing K^+ for transport to shoots (Jeschke, 1984).

A high shoot selectivity for K^+ over Na^+ can be seen in seashore paspalum and manilagrass. Shoot K^+/Na^+ ratios were higher in these 2 grasses than in the others at 400 *mM* NaCl. However, St. Augustinegrass and Tifway bermudagrass were also selective for K^+ over Na^+, as can be seen by comparing shoot K^+/Na^+ ratios with that of the nutrient solution at 400 *mM* NaCl (Table 6).

Seashore paspalum must rely exclusively on K^+/Na^+ selectivity of the roots to maintain Na^+ exclusion from the shoots. However, manilagrass and Tifway bermudagrass were also able to limit shoot Na^+ and Cl^- concentrations by the presence of active leaf salt glands, secreting 0.50 and 0.46 mmol g^{-1} leaf dry wt. of Na^+ per week, respectively, when grown at 200 *mM* NaCl. The large amounts of Na^+ (and also Cl^-) secreted can readily be seen when compared to the total tissue Na^+ present in the leaves at 200 *mM* NaCl, which was 0.33 and 0.48 mmol g^{-1} leaf dry wt. in manilagrass and Tifway bermudagrass, respectively. Manilagrass secreted 150% of total Na^+ present in leaf tissue on a weekly basis.

Though salt glands were also present in Japanese lawngrass, they were only half as efficient as those in manilagrass and Tifway bermudagrass, secreting about half the Na^+ and Cl^- per leaf weight. As a consequence, Japanese lawngrass did not limit shoot Na^+ and Cl^- levels as effectively, resulting in relatively high shoot ion levels at intermediate salinities. When expressed on a tissue water basis shoot Na^+ and Cl^- levels were extremely high (Figs. 22 and 23), which was partly due to the very low shoot tissue succulence of this grass.

Centipedegrass accumulated Na^+, and especially Cl^-, to high levels in the shoots at as low as 100 *mM* NaCl. As in Japanese lawngrass, Na^+ and Cl^- levels on a tissue water basis were extremely high, in fact, the tissue water Cl^- concentration in centipedegrass at 100 *mM* NaCl was higher than in any other grass at any salinity. As there were no salt glands present in centipedegrass, poor root ion selectivity was responsible for ion excesses. Both centipedegrass and Japanese

lawngrass showed evidence of Na^+ and Cl^- toxicity, suffering moderate to severe leafburn and shoot dieback at only 100 and 200 *mM* NaCl, respectively.

St. Augustinegrass responded differently to salinity than did the other grasses, and was in many ways similar to the highly salt tolerant dicotyledonous halophytes. Shoot growth was stimulated at intermediate salinity levels in St. Augustinegrass. Shoot growth stimulation under moderate salinity is often used as a criteria in the definition of a true halophyte (Weimberg, 1986), and is typical of a number of highly salt tolerant halophytes, but within the Poaceae has been reported to occur only in the *Puccinellia* species (Munns *et al.*, 1983b).

The high shoot growth rates in St. Augustinegrass were associated with high shoot Na^+ and Cl^- levels and tissue succulence. Salt accumulation in shoots is generally associated with tissue succulence in salt tolerant halophytes, due to efficient compartmentation of salts in large vacuoles of the mesophyll cells, coupled with osmotic influx of water (Kramer, 1984). Shoot Na^+ and Cl^- levels in St. Augustinegrass were similar to those found in salt-accumulating dicotyledonous halophytes of the families Chenopodiaceae, Plantaginaceae, and Caryophyllaceae (Storey *et al.*, 1977; Gorham *et al.*, 1980). There are a few halophytic grasses known to accumulate Na^+ and Cl^- to similar levels, notably *Spartina* spp. and *Triglochin maritima* (Gorham *et al.*, 1980). Shoot K^+/Na^+ ratios in St. Augustinegrass at high salinity were very low (0.17), which again is similar to those found in the Chenopodiaceae and Caryophyllaceae (Gorham *et al.*, 1980) which have

relatively low affinities for K^+ coupled with high Na^+ uptake (Wyn Jones, 1981).

When tissue concentration of NaCl exceeds about 200 *mM* ion compartmentation within the vacuole becomes necessary to avoid enzyme deactivation and subsequent cell death. Under these conditions, the maintenance of osmotic equilibrium across the tonoplast requires the accumulation in the cytoplasm of nontoxic organic solutes, or "compatible solutes" , the most likely candidates being glycinebetaine and proline (Gorham *et al.*, 1985b; Wyn Jones, 1981).

A number of methods have been used to obtain evidence that the location of glycinebetaine and proline is predominately in the cytoplasm (Gorham and Wyn Jones, 1983; Leigh *et al.*, 1981). If this is assumed to be true, and if a further assumption is made that the cytoplasmic volume in mature mesophyll cells in the grasses studied is approximately 10% of total cell volume, estimates of the relative contributions of glycinebetaine and proline to the osmotic adjustment of the cytoplasm can be made (Table 9).

In Table 9, shoot tissue concentrations of glycinebetaine and proline are first converted into molar concentrations on a tissue water basis. The estimated contributions to cytoplasmic osmotic pressure can then be calculated, assuming that these compounds are located exclusively in the cytoplasm (Leigh *et al.*, 1981).

On this basis the cytoplasmic concentrations of glycinebetaine and proline in Tifway bermudagrass, manilagrass, seashore paspalum, and Japanese lawngrass would be sufficient for complete cytoplasmic osmotic adaptation, accomodating all the increase in cytoplasmic osmotic

Table 9. The possible osmotic contributions of glycinebetaine and proline to cytoplasmic osmotic adjustment in grasses grown under 400 *mM* NaCl. (Centipedegrass grown under 200 *mM* NaCl).

Grass	Shoot Tissue Content (μmol g^{-1} drw wt.) or (mM)[y]				[z]Estimated contribution to cytoplasmic osmotic adjustment (mOsmol kg^{-1})
	Glycinebetaine		Proline		
Ber.	196	(109)	208	(115)	2240
Manl.	143	(95)	16	(10)	1050
Pasp.	200	(61)	38	(12)	730
Saug.	151	(39)	55	(14)	530
Japn.	123	(77)	40	(25)	1020
Cent.	4	(2)	20	(10)	120

[y]Tissue in brackets are concentrations calculated on a tissue water basis.

[z]Estimate assuming glycinebetaine and proline to be concentrated in cytoplasm occupying 10% of the cell volume.

pressure above a basal level of 300-400 mOsmol kg^{-1}. However, it should be pointed out that the large tissue water concentrations of glycinebetaine and proline in Tifway bermudagrass, manilagrass, and Japanese lawngrass are partly due to the concentrating effect of low fresh wt./dry wt. ratios. Though the levels in St. Augustinegrass would contribute substantially to osmotic adjustment, they would still be about 200 *mM* short of fully adjusting cytoplasmic osmotic potentials at 400 *mM* NaCl. Glycinebetaine and proline levels in centipedegrass were too low to affect any significant osmotic adjustment of the cytoplasm. This lack of compatible solutes may mean that centipedegrass was unable to compartmentalize ions within shoot vacuoles. This, coupled with high Na^+ and Cl^- shoot concentrations due to an inability to restrict these ions from the shoots, may be responsible for the salt sensitivity of centipedegrass.

CHAPTER VI

SALT TOLERANCE OF THE COASTAL SALT MARSH GRASS *SPOROBOLUS VIRGINICUS* (L.) KUNTH

ABSTRACT

Sporobolus virginicus (L.) Kunth was grown under solution culture in salinities of up to 450 *mM* NaCl. Growth responses, leaf water and osmotic relations, and solute contents were determined. Shoot growth was stimulated by intermediate salt levels, concurrent with both an accumulation of Na^+ and Cl^- in shoots and a slight increase in shoot succulence. Root growth was stimulated at salinities up to 450 *mM* NaCl. Osmotic adjustment of shoots was predominantly due to Na^+, Cl^-, and soluble carbohydrate accumulation, though shoot dehydration played a role at high salinity. Shoot Na^+ and Cl^- accumulation was tightly controlled, not exceeding levels required for osmotic adjustment. Massive secretion of Na^+ and Cl^- by leaf salt glands was no doubt primarily responsible. Shoots had a high affinity for K^+ at high salinity, maintaining fairly constant K^+ concentrations with increasing salinity. Increasing NaCl stimulated the accumulation of K^+ in roots, which may have acted as a reservoir of K^+ for shoots at high salinity. Glycinebetaine, and to a lesser extent proline, accumulated in shoot tissues with increasing salinity. Accumulation was closely associated with increases in shoot sap osmolalities. It is proposed that glycinebetaine may act as a compatible solute in *Sporobolus virginicus*,

as levels were sufficiently high to effect total osmotic adjustment of the cytoplasm at high salinity, assuming localization in the cytoplasm.

INTRODUCTION

Soil salinity places major constraints on plant growth in arid and maritime regions. Efforts in breeding for salt tolerance have been hindered by an inadequate understanding of the salt tolerance mechanisms in higher plants. Information regarding salt tolerance mechanisms of monocotyledonous halophytes is relatively lacking, as the majority of physiological studies have focused on dicotyledonous halophytes.

The adaptations of halophytes (plants that occur naturally under saline conditions) to salinity differ from those of glycophytes (Stewart and Ahmed, 1983). Growth of halophytes, especially dicotyledonous, is generally stimuluated by intermediate salt levels (Flowers *et al.*, 1977). Under saline conditions leaves of halophytes accumulate NaCl for osmotic adjustment, and often increase in succulence (Flowers and Yeo, 1986). Halophytes accumulate NaCl to levels which are inhibitory to enzyme activity, necessitating efficient compartmentation of saline ions in the vacuole (Flowers *et al.*, 1977; Flowers, 1985).

Under these conditions compatible organic solutes which do not interfere with enzyme activity are required to balance osmotic pressure with that of the vacuole. There is substantial evidence that glycinebetaine and proline fulfill this role in many halophytes. Evidence is listed below.

1. Glycinebetaine accumulation in shoots is highly correlated with increases in sap osmolality above a basal level of 220-400 mOsmol kg^{-1} (Wyn Jones, 1984; Wyn Jones and Storey, 1981).
2. Glycinebetaine and proline are nontoxic to metabolic functions at concentrations of 1 M and above (Wyn Jones, 1984; Stewart and Lee, 1974).
3. Glycinebetaine and proline are located primarily within the cytoplasm, as determined by a number of techniques (Hall *et al.*, 1978; Gorham and Wyn Jones, 1983; Leigh *et al.*, 1981).
4. Glycinebetaine and proline affect specific ion fluxes across membranes and may stabilize membrane integrity under salt stress (Jolivet *et al.*, 1983).
5. Glycinebetaine and proline stabilize enzymes against perturbation by salinity (Pollard and Wyn Jones, 1979). Glycinebetaine and proline protected phosphoenolpyruvate carboxylase extracted from *Cynodon dactylon* and *Sporobolus pungens* from inactivation when exposed to salinity in vitro and lowered the K_{mPEP}, resulting in increased activity of the enzyme (Manetas *et al.*, 1986).

Excess ion accumulation inhibits growth in many glycophytes. A comparison of the relative degrees of growth inhibition among closely related species sometimes reveals that salt tolerance is associated with Na^+ exclusion (Weimberg, 1986). Osmotic adjustment is often aided on a whole cell basis by sugars, which is typical of many monocotyledonous plants, including grasses (Yeo, 1983; Gorham *et al.*, 1980).

The purpose of this research was to determine the physiological and growth responses of *Sporobolus virginicus* (L.) Kunth to salinity in

an attempt to elucidate salt tolerance mechanisms used. *S. virginicus* is a perennial, coastal salt marsh grass that is adapted to sandy or muddy seashores and saline marshes, forming extensive colonies (Chase, 1971). Plants in this experiment were grown from rhizome segments taken from a beach on the southeastern end of the island of Oahu, Hawaii.

MATERIALS AND METHODS

Rhizomes of *Sporobolus virginicus* (L.) Kunth were collected from plants growing near the shore of a beach on the south side of Oahu, Hawaii. Rhizomes were trimmed to a length of 5 cm and planted into 9 cm diameter by 6 cm deep plastic pots having coarse screen bottoms, which were filled with no. 12 silica sand. Pots were placed in lids and suspended over plastic tubs containing constantly aerified, modified Hoagland no. 2 solution in deionized water (Hoagland and Arnon, 1950). The solution was modified by supplying 2 mg Fe L^{-1} as Fe-EDDHA chelate (Ciba-Geigy Sequestrene 138), and by maintaining a 1 *mM* concentration of NaCl in the control solution, as *S. virginicus* employs the C_4 photosynthetic pathway which requires Na^+ as an essential micronutrient (Brownwell and Crossland, 1972). Plants were grown in a glasshouse from October, 1987 to May, 1988. Plants were trimmed every 2 weeks at a height of 10 cm throughout the period. *S. virginicus*, a slow growing perennial grass, required over 2 months to become densely established in the pots before salinity treatments were initiated on January 21, 1988. Sodium chloride concentrations were increased daily by increments of 50 *mM* (2.9 g L^{-1}) until final levels of 1, 150, 300, and 450 *mM* were

reached. Nutrient solutions were kept at a constant volume and changed weekly to maintain approximately constant salinity levels.

Shoot harvests were begun on February 18, for a total of 7 harvests over a period of 3 months. Following the final harvest, roots growing through the pot screens into the nutrient solution were clipped. Both shoots and roots were washed in deionized water for 20 seconds. Shoots were allowed to dry after rinsing before being clipped. Clipped shoots and roots were dried at 70°C for 48 hours for dry weight determination.

Immediately prior to the second harvest, a small amount of unrinsed shoots were clipped at each salinity level to determine the amount of salt secretion by salt glands, measured as the difference in ion contents between unrinsed and rinsed shoots grown under the same salinity. At the second harvest rinsed shoots (air-dried) were clipped and immediately placed in air-tight bottles for fresh weight determination, then dried at 70°C as before to determine shoot fresh wt./dry wt. ratios.

Also prior to the second harvest a small amount of rinsed shoots were placed in both air-tight microcentrifuge tubes and vials for determination of leaf sap osmolality and proline, respectively, and immediately frozen in dry ice. Leaf sap osmolality and proline were determined as described in Chapter V.

For determination of total soluble carbohydrates, dried, ground shoots were extracted in 10 ml of boiling, 80% ethanol for 30 minutes, filtered, and brought to 100 ml volume. The method of Dubois *et al.*, 1956, was used, with glucose as standard. The inorganic ions Na^+, K^+,

Ca^{2+}, Mg^{2+}, and Cl^{-}, and the betaines glycinebetaine and trigonelline were determined as described in Chapter V.

RESULTS

Growth

Shoot growth was stimulated by NaCl, reaching an optimum growth rate of 1.4 g dry wt./week/pot at about 150 *mM* NaCl (Fig. 36). Shoot growth rates for *S. virginicus* were higher across all salinities than in the other grasses previously studied (cf. Chapter V). This could be due in part to the higher cutting height (4 cm) maintained for *S. virginicus* as compared to the other grasses (2.5 cm). Shoot growth followed the same trend as St. Augustinegrass, both having growth optimums at approximately 150 *mM* NaCl (cf. Chapter V).

Root growth increased linearly with increasing salinity (Fig. 37). Root length exceeded 4 feet in plants grown at high salinity (data not shown). Stimulated root growth with increasing salinity resulted in large increases in root/shoot ratios. The root/shoot ratio increased from 0.50 at 1 *mM* NaCl to 2.22 at 450 *mM* NaCl. An increase in root/shoot ratios with increasing salinity has been reported to occur in bermudagrass (Younger and Lunt, 1967) and in seashore paspalum (Dudeck and Peacock, 1985). Salinity tolerance was related to greater root growth within selected lines of four grass species (Ashraf *et al.*, 1989).

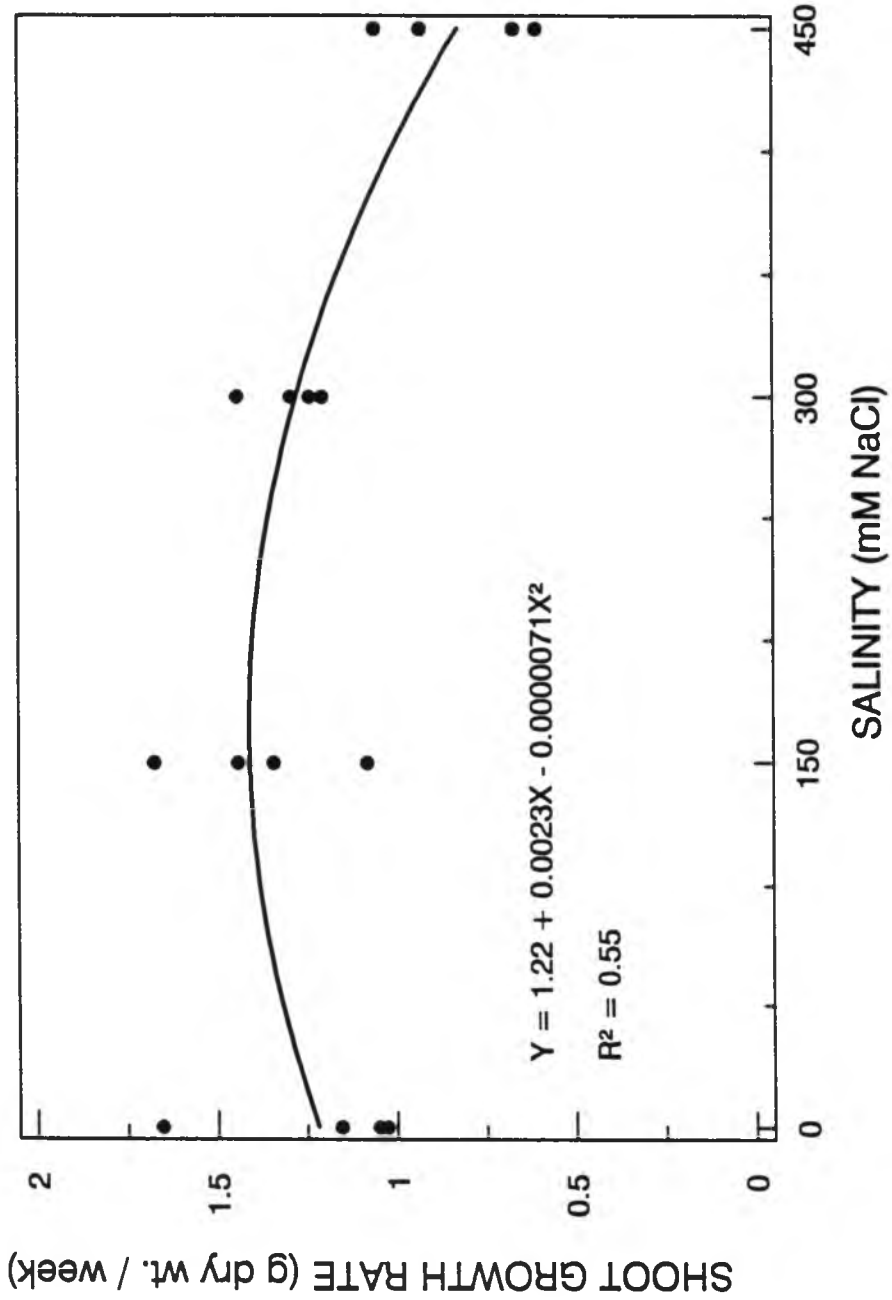


Figure 36. Shoot growth rate, expressed as g dry wt./pot/week, as influenced by NaCl level.

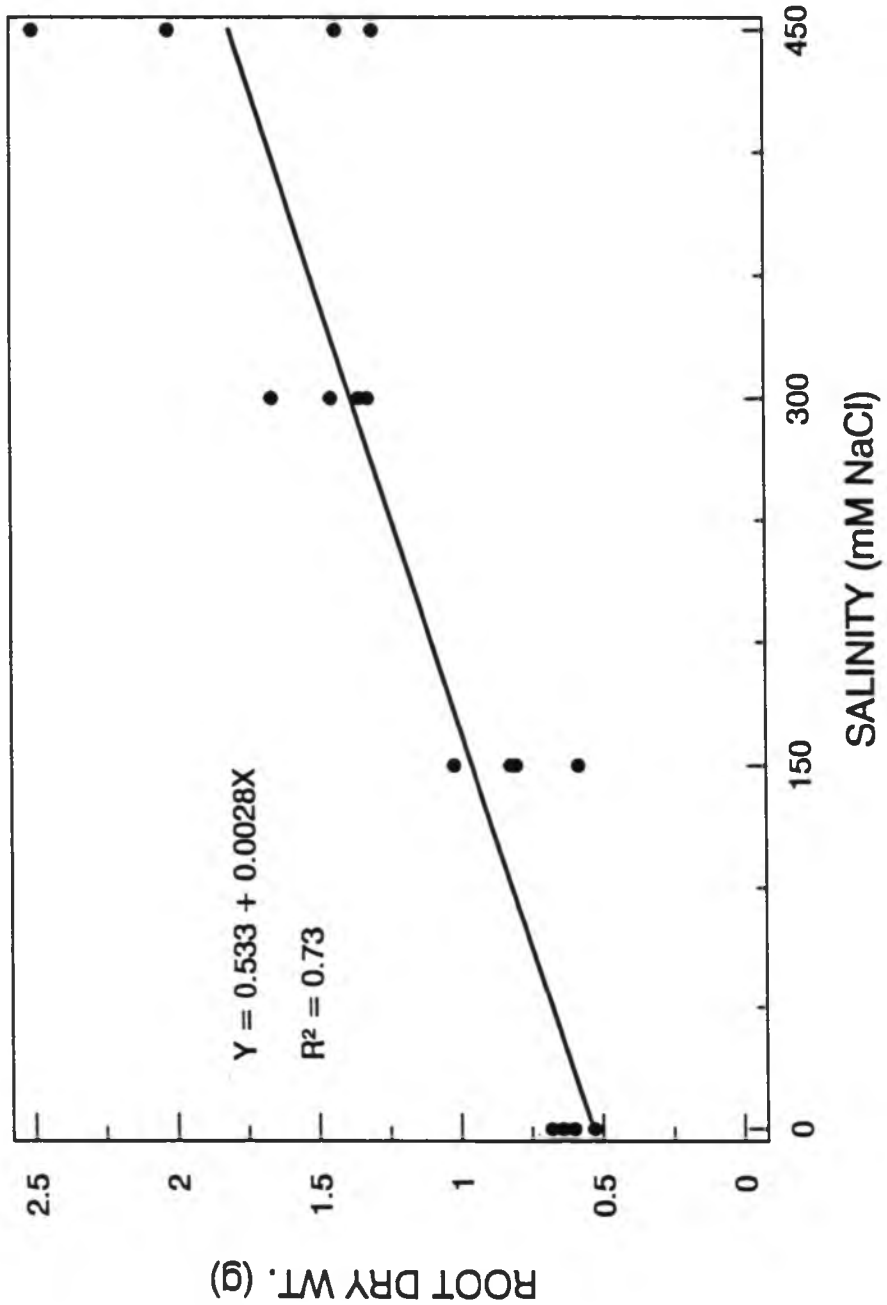


Figure 37. Root dry weight as influenced by NaCl level.

Ion Relations

Shoot Na^+ and Cl^- increased with increasing salinity (Figs. 38-39). Shoot Na^+ reached a level of 0.9 mmol g^{-1} dry wt., which was about twice the level of Cl^- in shoots. These Na^+ levels are similar to those of other salt tolerant grasses at comparable salinities (Ahmad *et al.* 1981) and to bermudagrass, a member of the Chloridoideae subfamily as is *Sporobolus* spp. (cf. Chapter 3). However, they are low when compared to Na^+ accumulators such as *Spartina townsendii* (Storey and Wyn Jones 1978a), a halophyte of the Poaceae, and to dicotyledonous halophytes in general (Gorham, 1980). Root Na^+ concentrations were similar to those of shoot Na^+ at higher salinity (Fig. 40). However, root Cl^- concentrations, while initially lower than shoot Cl^- concentrations, surpassed shoot concentrations at higher salinities (Fig. 41), indicating that Cl^- transport to shoots was restricted.

Shoot K^+ levels remained relatively stable across salinity, averaging 0.4 mmol g^{-1} dry wt. (Fig. 42). Similar responses for shoot K^+ at high salinity have been observed in the grass halophyte *Spartina townsendii* (Storey and Wyn Jones 1978) and in bermudagrass (cf. Chapter 3), which belong to the same subfamily as *Sporobolus* (Chloridoideae). This contrasts with the large drop in shoot K^+ with increasing salinity frequently observed in dicotyledonous halophytes (Albert, 1975). A preferential shoot K^+ uptake at higher salinities is evident when the shoot K^+/Na^+ ratios are compared with those of the nutrient solution (Table 10). The shoot K^+/Na^+ ratio is twice that of the nutrient solution at 1 *mM* NaCl, and 57 times higher at 450 *mM* NaCl. Shoot K^+ selectivity is also indicated by increasing K^+ selectivity ratios

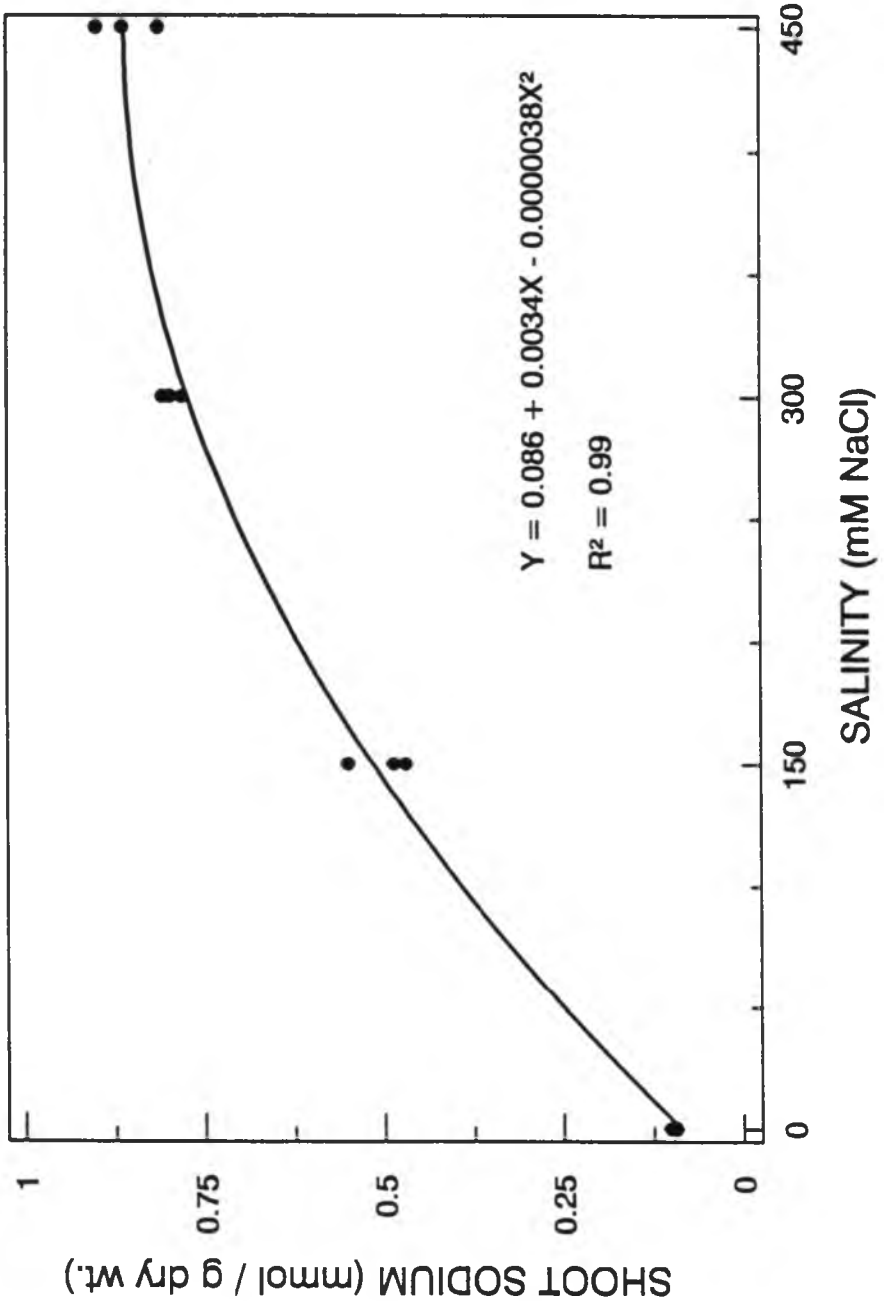


Figure 38. Shoot Na^+ concentration, expressed on a dry weight basis, as influenced by NaCl level.

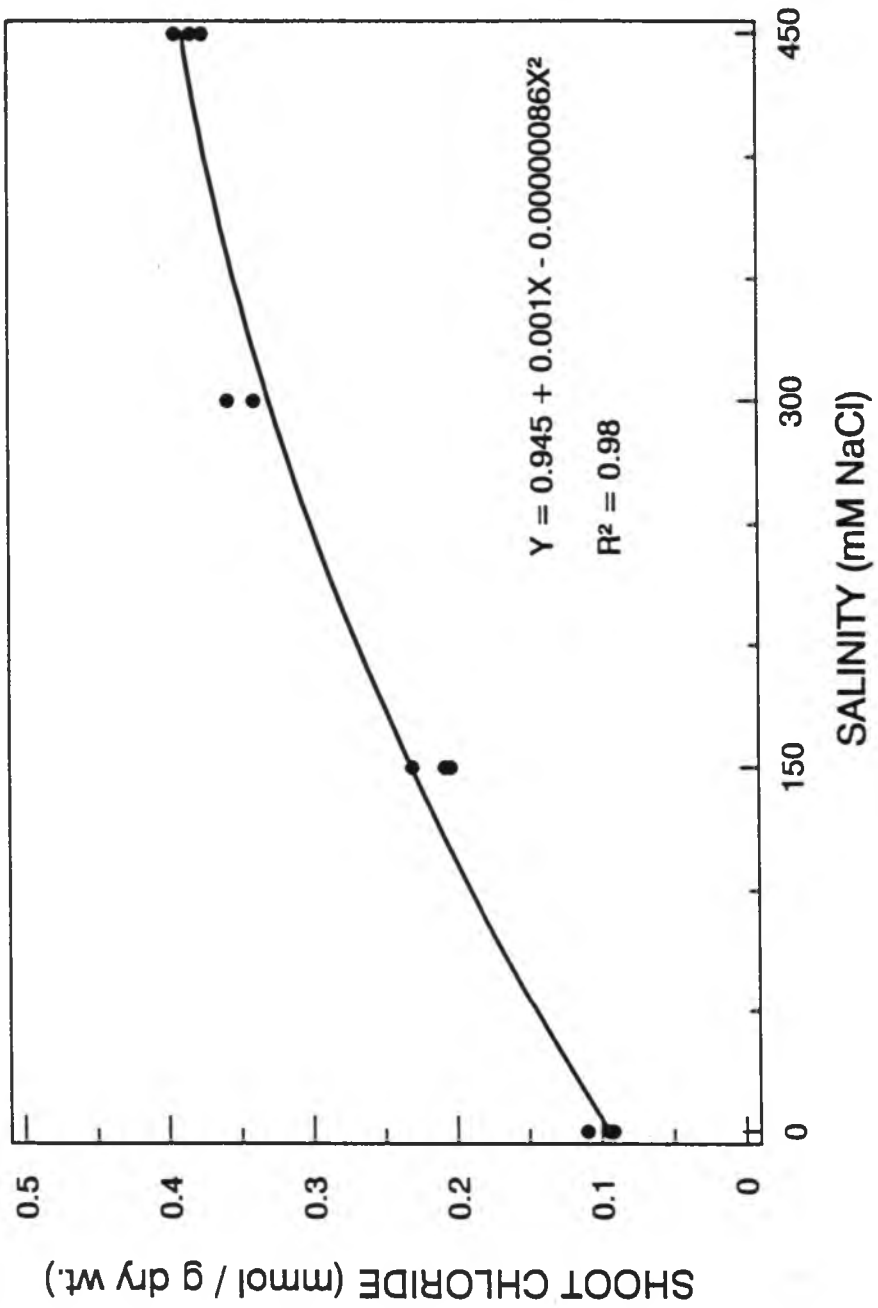


Figure 39. Shoot Cl^- concentration, expressed on a dry weight basis, as influenced by NaCl level.

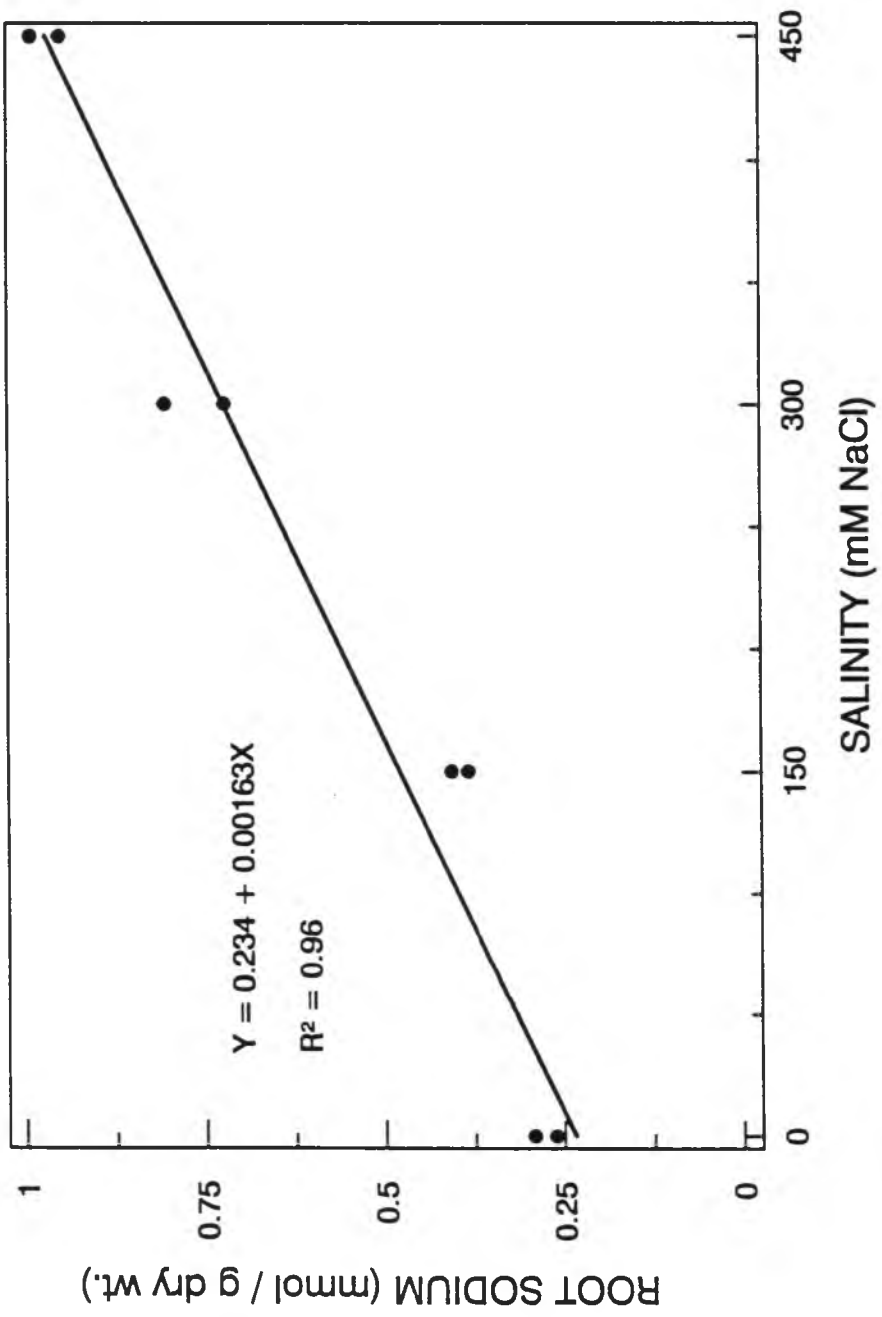


Figure 40. Root Na^+ concentration, expressed expressed on a dry weight basis, as influenced by NaCl level.

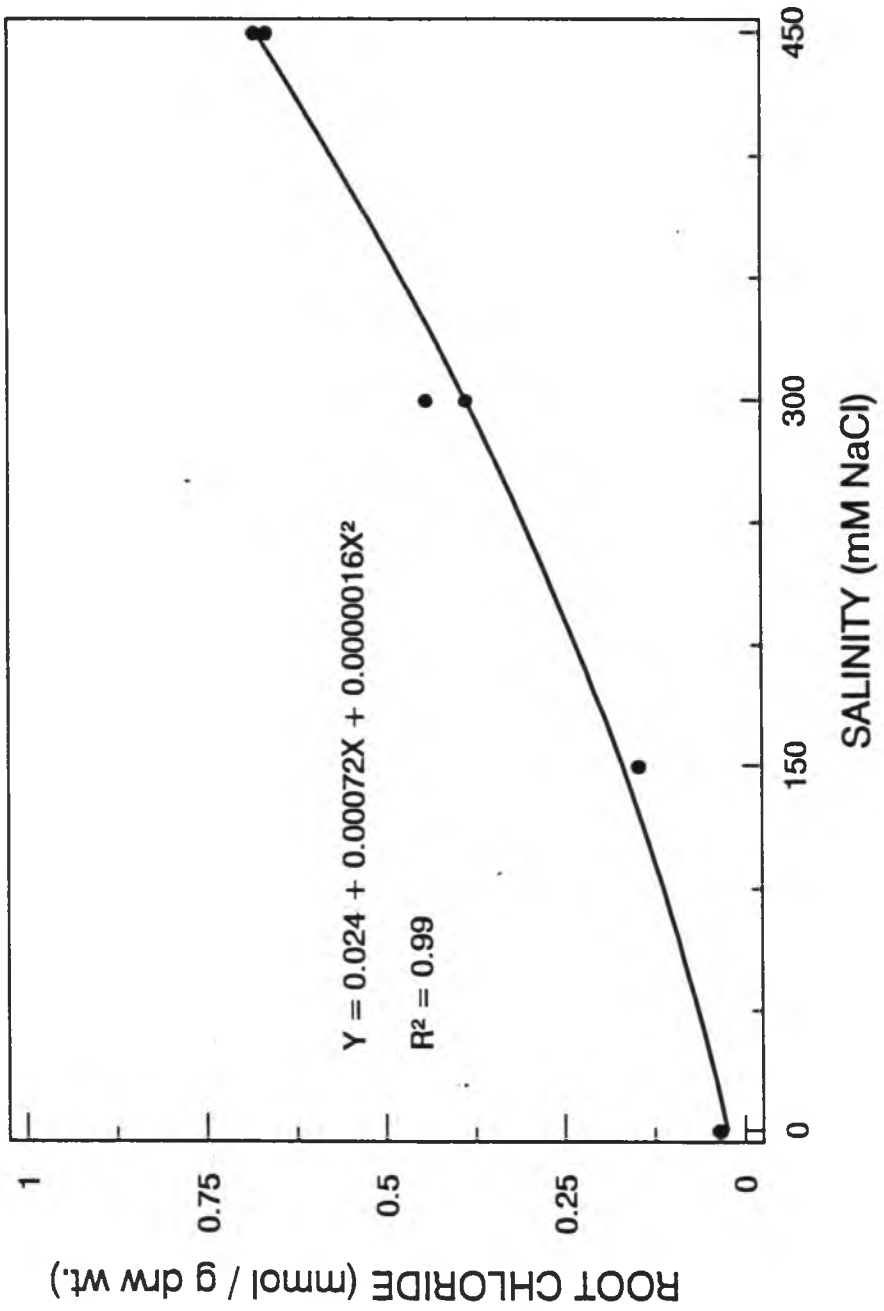


Figure 41. Root Cl^- concentration, expressed expressed on a dry weight basis, as influenced by NaCl level.

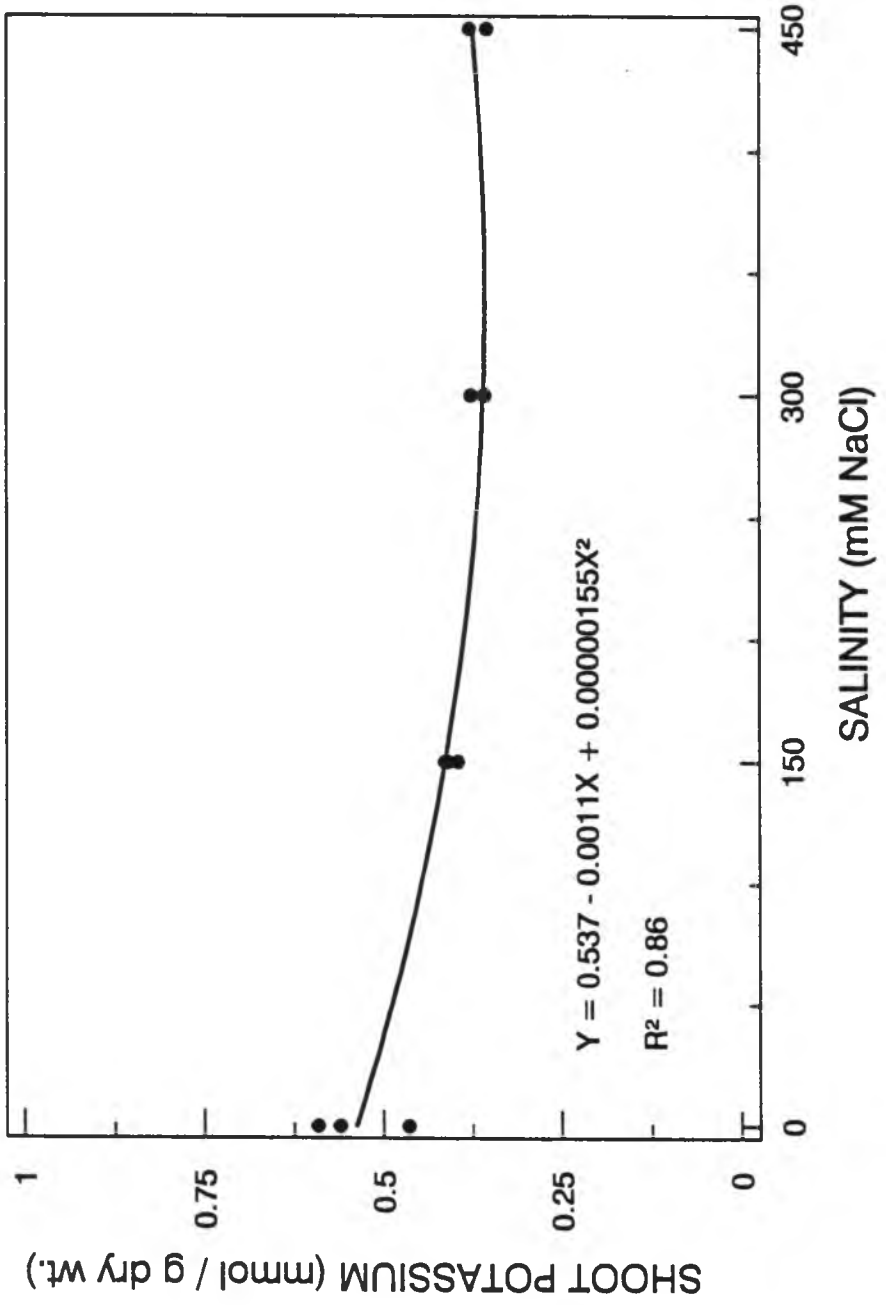


Figure 42. Shoot K^+ concentration, expressed on a dry weight basis, as influenced by NaCl level.

Table 10. Shoot K^+/Na^+ ratios, root media K^+/Na^+ ratios, and shoot selectivity ratios ($S_{K,Na}$) for K^+ at different salinities. $S_{K,Na}$=[K in plant][Na in medium]/[Na in plant][K in medium].

Salinity	Shoot K^+/Na^+	Media K^+/Na^+	Shoot $S_{K,Na}$
1	5.9	3.000	2.0
150	0.8	0.020	40.9
300	0.5	0.010	46.1
450	0.4	0.007	65.3

($S_{K,Na}$=[K in plant][Na in medium]/[Na in plant][K in medium]) (Pitman, 1969).

The K^+ concentration in roots actually increased with increased salinity (Fig. 43). A NaCl stimulation of K^+ uptake in roots has been reported to occur in the halophytes *Triglochin maritima* (Jefferies, 1973), *Suaeda monoica* (Storey and Wyn Jones, 1979), and *Spartina townsendii* (Storey and Wyn Jones, 1978a). Roots with a high affinity for K^+ in the presence of NaCl may be directly involved in sustaining shoot selectivity for K+ at high salinities.

Shoot Ca^{2+} and Mg^{2+} levels were little affected by increasing salinity (Figs. 44-45). Shoot Ca^{2+} levels rose slightly at 150 *mM* NaCl, then declined. Root Ca^{2+} levels were initially higher than those of the shoots, but rapidly decreased to less than the shoots at high salinity (Fig. 46). Root Mg^{2+} levels were much lower than in shoots across all salinities (Fig. 47). Higher concentrations of Ca^{2+} and Mg^{2+} in shoots than in roots may be an indication that minimum shoot levels for metabolism are being maintained. Calcium is known to have a beneficial effect on the salt tolerance of many plants, possibly by maintaining membrane integrity (Huq and Larher, 1984).

The levels of soluble carbohydrates in shoots increased with increasing salinity, reaching 380 μmol g^{-1} dry wt. at 450 *mM* NaCl (Fig. 48). This is similar to levels reported in the grass halophytes *Puccinellia maritima* and *Spartina townsendii* (Briens and Larher, 1982).

Sporobolus virginicus actively secreted salt, as evidenced by dense accumulations of salt crystals on both adaxial and abaxial leaf surfaces of plants grown in saline media. Salt secretion was determined

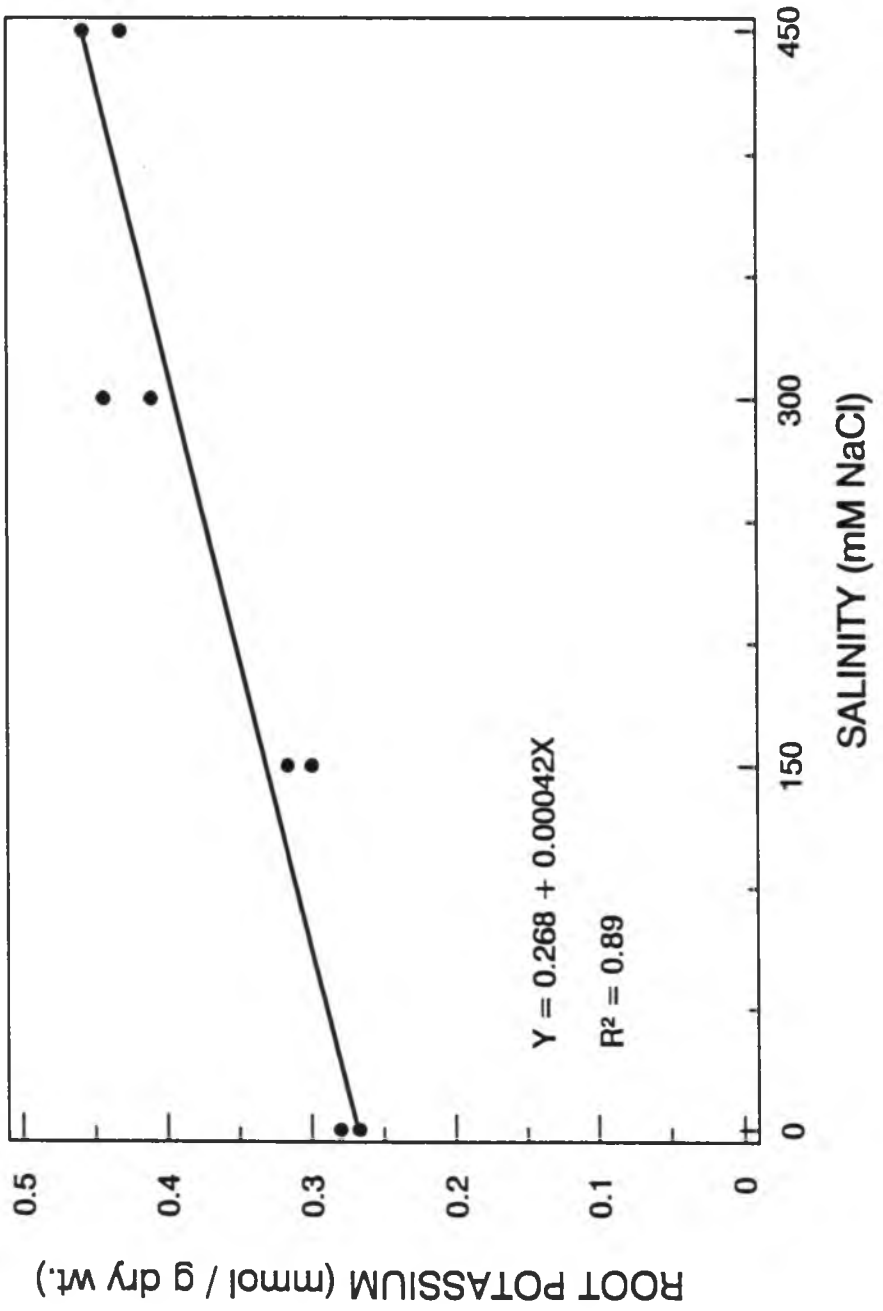


Figure 43. Root K^+ concentration, expressed on a dry weight basis, as influenced by NaCl level.

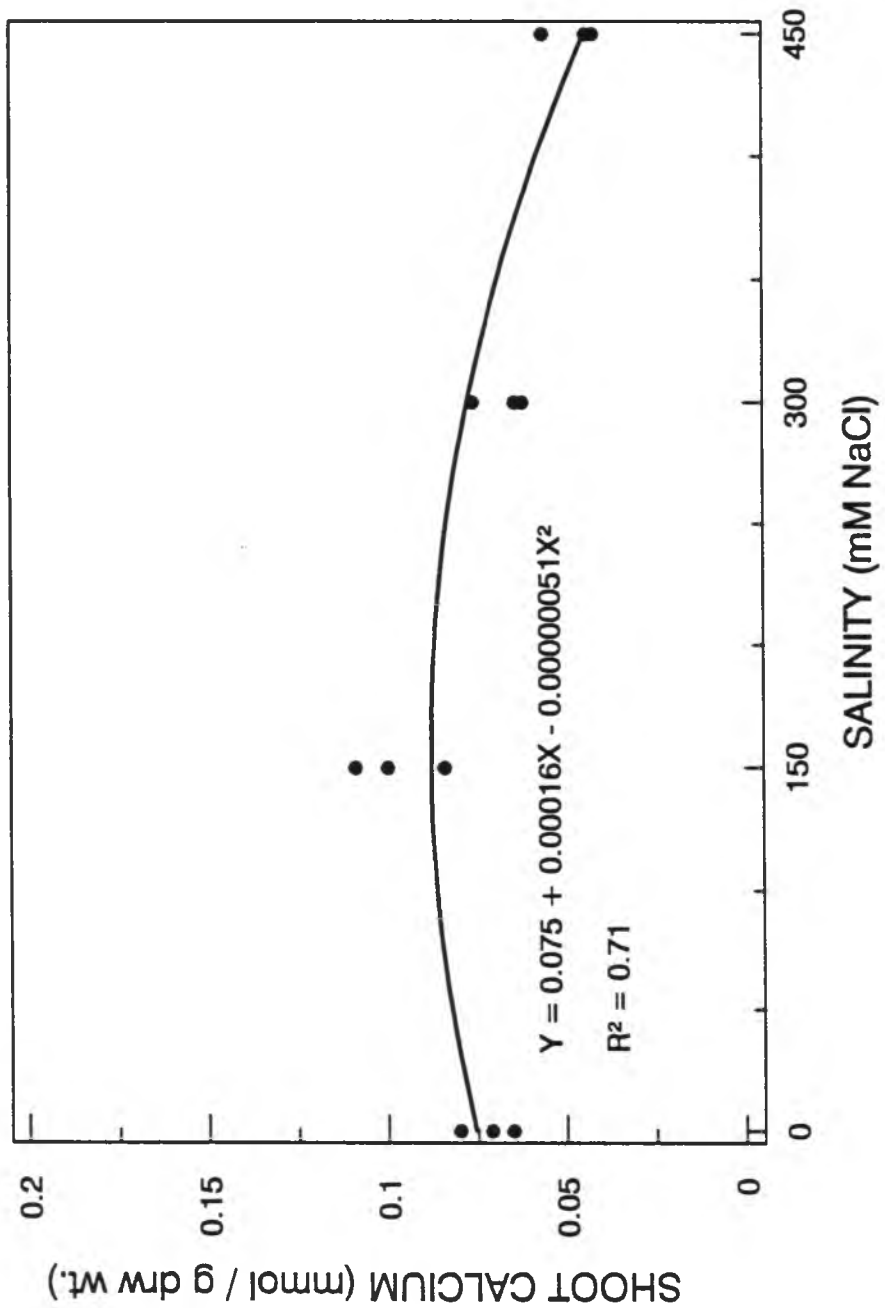


Figure 44. Shoot Ca^{2+} concentration, expressed on a dry weight basis, as influenced by NaCl level.

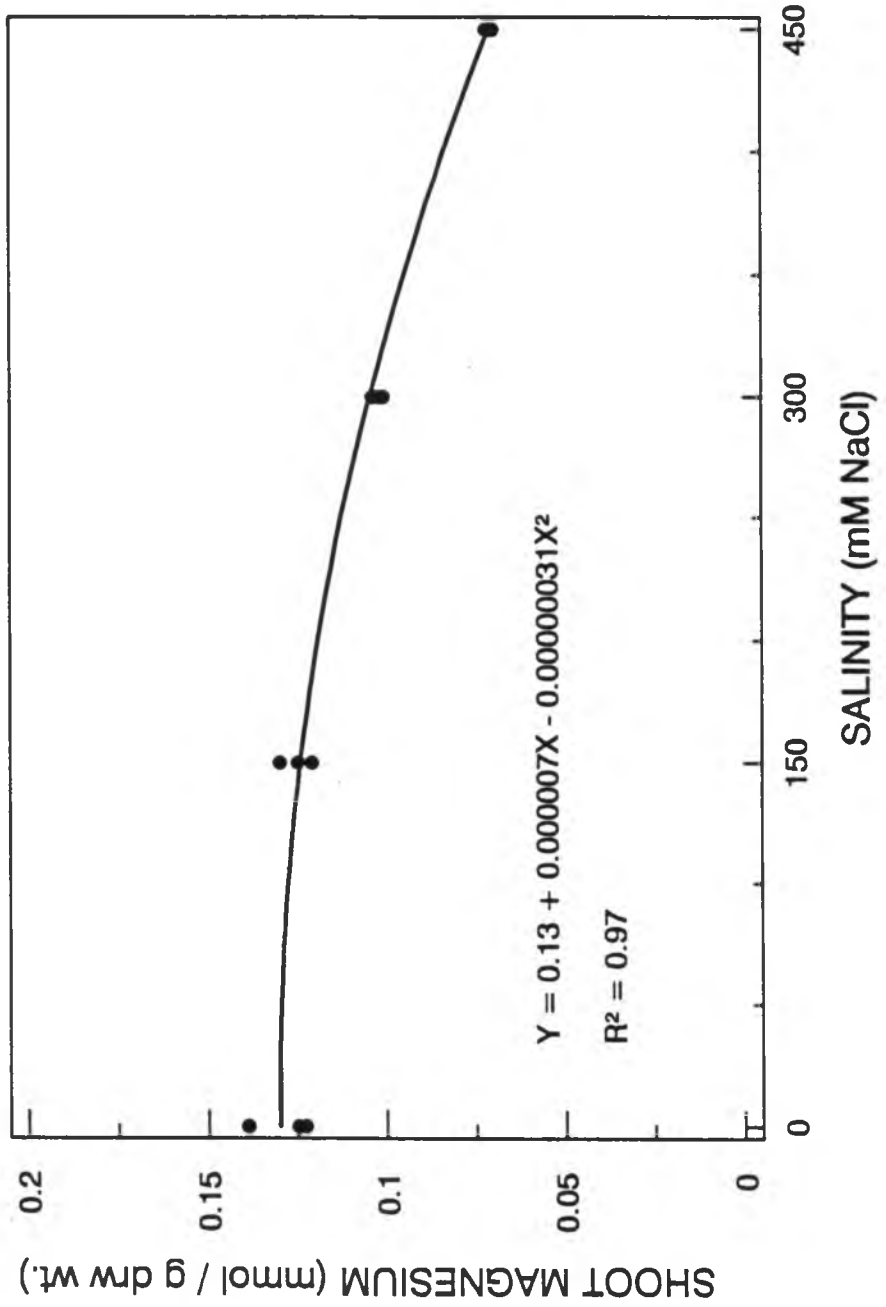


Figure 45. Shoot Mg^{2+} concentration, expressed on a dry weight basis, as influenced by NaCl level.

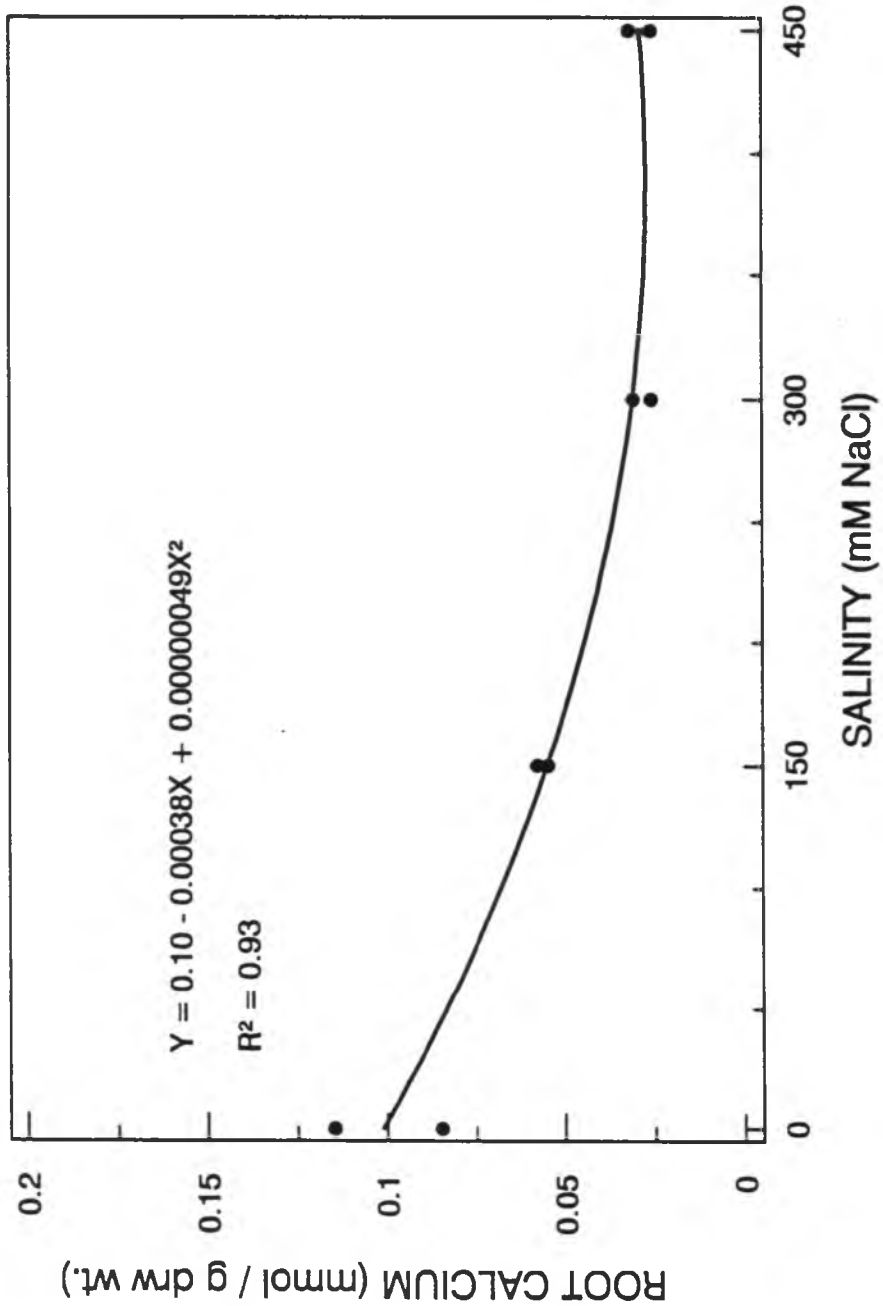


Figure 46. Root Ca^{2+} concentration, expressed on a dry weight basis, as influenced by NaCl level.

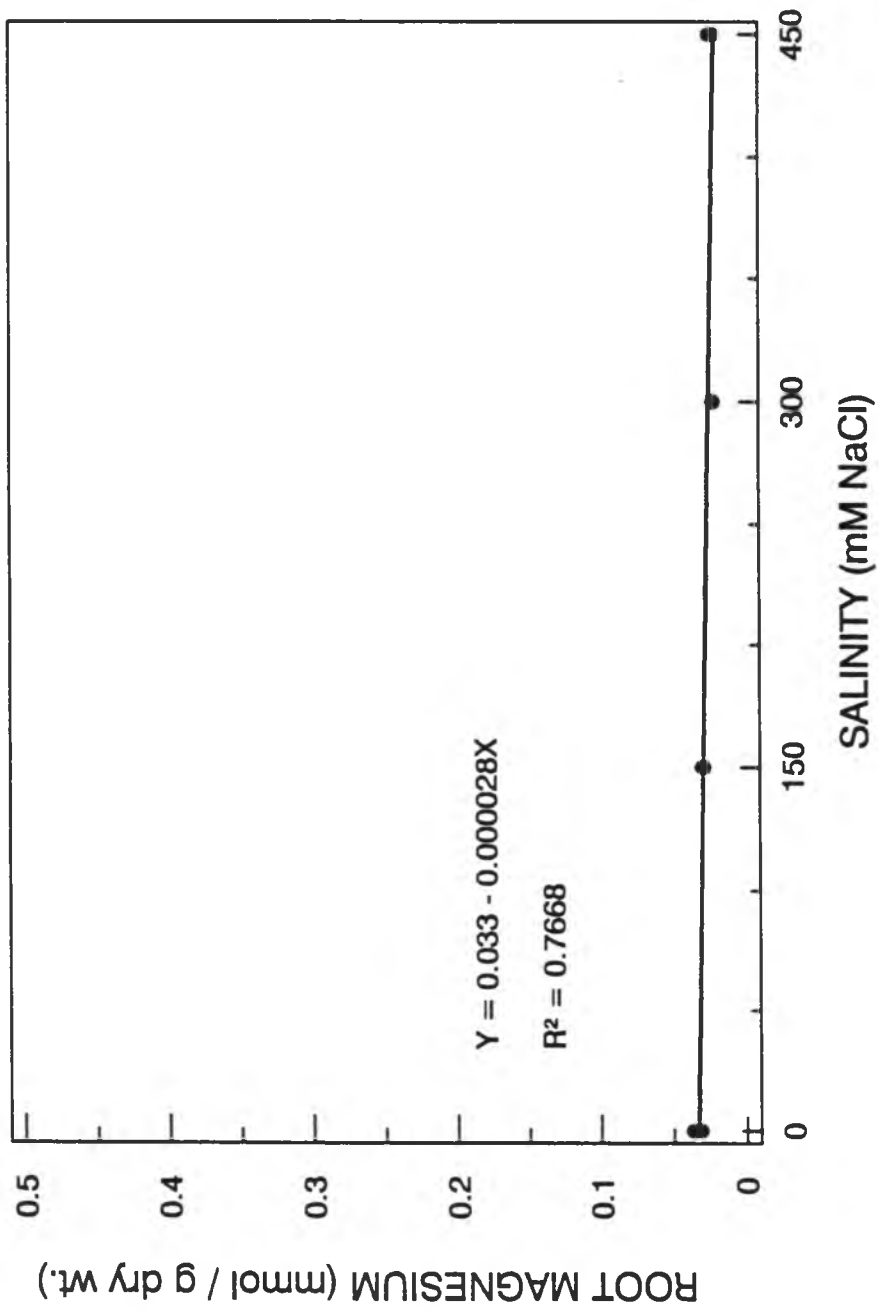


Figure 47. Root Mg^{2+} concentration, expressed on a dry weight basis, as influenced by NaCl level.

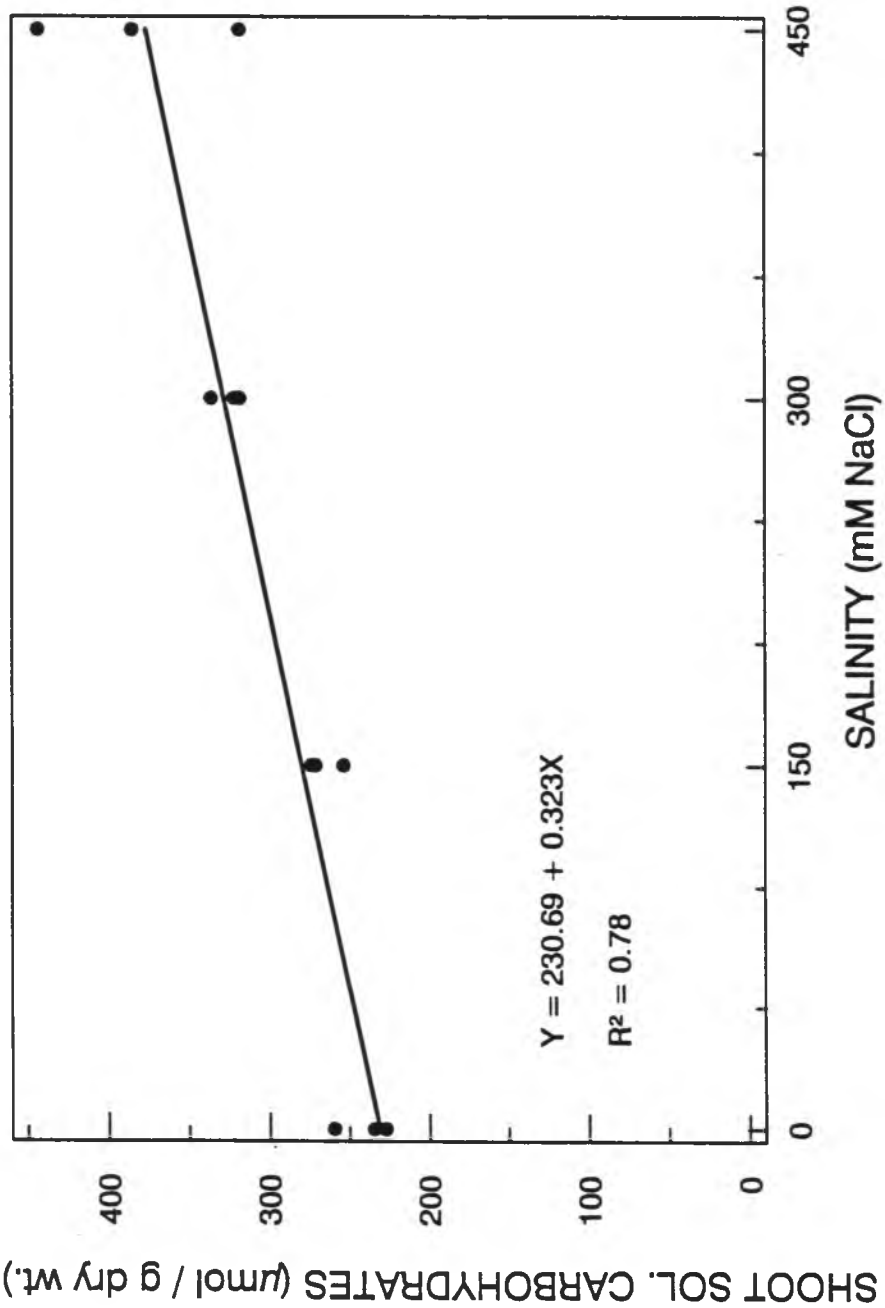


Figure 48. Shoot soluble carbohydrate concentration, expressed on a dry weight basis, as influenced by NaCl level.

as the difference in ion concentrations between unrinsed and rinsed leaves (leaves rinsed in deionized water for 20 seconds) (Table 11). Secretion of Na^+ and Cl^- occured in all treatments. Sodium and Cl^- concentrations of unrinsed leaves reached a maximum of 32 mmol g^{-1} dry wt. Na^+, and 22 mmol g^{-1} dry wt. Cl^{-1}, respectively, when grown at 300 *mM* NaCl . This represents a massive secretion, being 39 times the amount of Na^+, and 61 times the amount of Cl^- found in rinsed leaves at 300 *mM* NaCl. This amount of secretion would play a major role in limiting Na^+ and Cl^- concentrations in shoots. Secretion of Na^+ and Cl^- were much greater than that found in bermudagrass or manilagrass (cf. Chapter V). Potassium was also secreted by *S. virginicus*, but not to the same extent as was Na^+ and Cl^-. Secretion of K^+ reached a maximum at 300 *mM* NaCl, and there was no secretion at 1 *mM* NaCl. Calcium and Mg^{2+} were not secreted at any salinity. Salt glands have also been reported to occur in *Sporobolus arenarius* (Gou.) Duv.-Jouv. (Lipschitz and Waisel, 1974). To date, all grasses known to possess salt glands belong to the subfamily Chloridoideae.

Osmotic Adaptation

S. virginicus adjusted osmotically, maintaining a more negative osmotic potential than that of the medium, though the differences became less pronounced with increasing salinity (Fig. 49). Shoot fresh wt./dry wt. ratios were relatively constant across salinity, indicating that loss of tissue succulence played only a minor role in osmotic adjustment (Fig. 50). This contrasts with more salt-sensitive grasses, in which osmotic adjustment under salt stress is due in large part to tissue

Table 11. Concentration of Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^- in rinsed and unrinsed leaves of *Sporobolus virginicus* grown under varying salinities. Differences between unrinsed and rinsed leaves represent salt secretion for one week.

	Ion (mmol g^{-1} dry wt.)									
	Na^+		K^+		Ca^{2+}		Mg^{2+}		Cl^-	
Salinity	rin.	unrin.	rin.	unrin.	rin.	unrin.	rin.	unrin.	rin.	unrin.
1	0.09	0.30**[z]	0.54	0.54NS	0.07	0.07NS	0.13	0.09NS	0.10	0.30**
150	0.49	10.42*	0.41	12.38*	0.10	0.07NS	0.12	0.09NS	0.22	5.47*
300	0.79	31.74**	0.36	14.35**	0.07	0.06NS	0.10	0.08NS	0.35	21.86**
450	0.85	24.82**	0.37	9.38*	0.05	0.04NS	0.07	0.06NS	0.39	19.24**

[z] Differences between unrinsed and rinsed values tested by paired Student's t test.

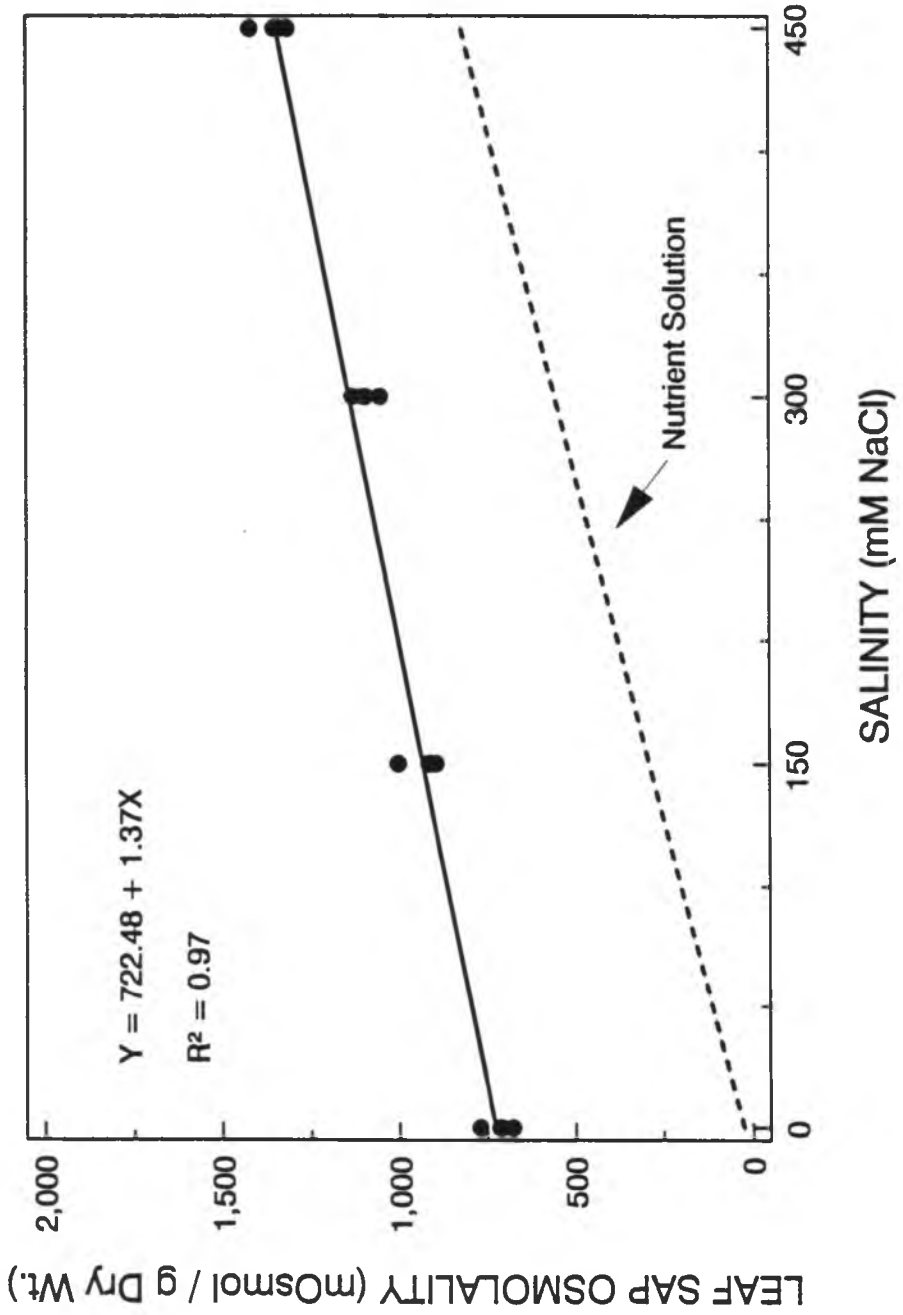


Figure 49. Leaf sap osmolality as influenced by NaCl level.

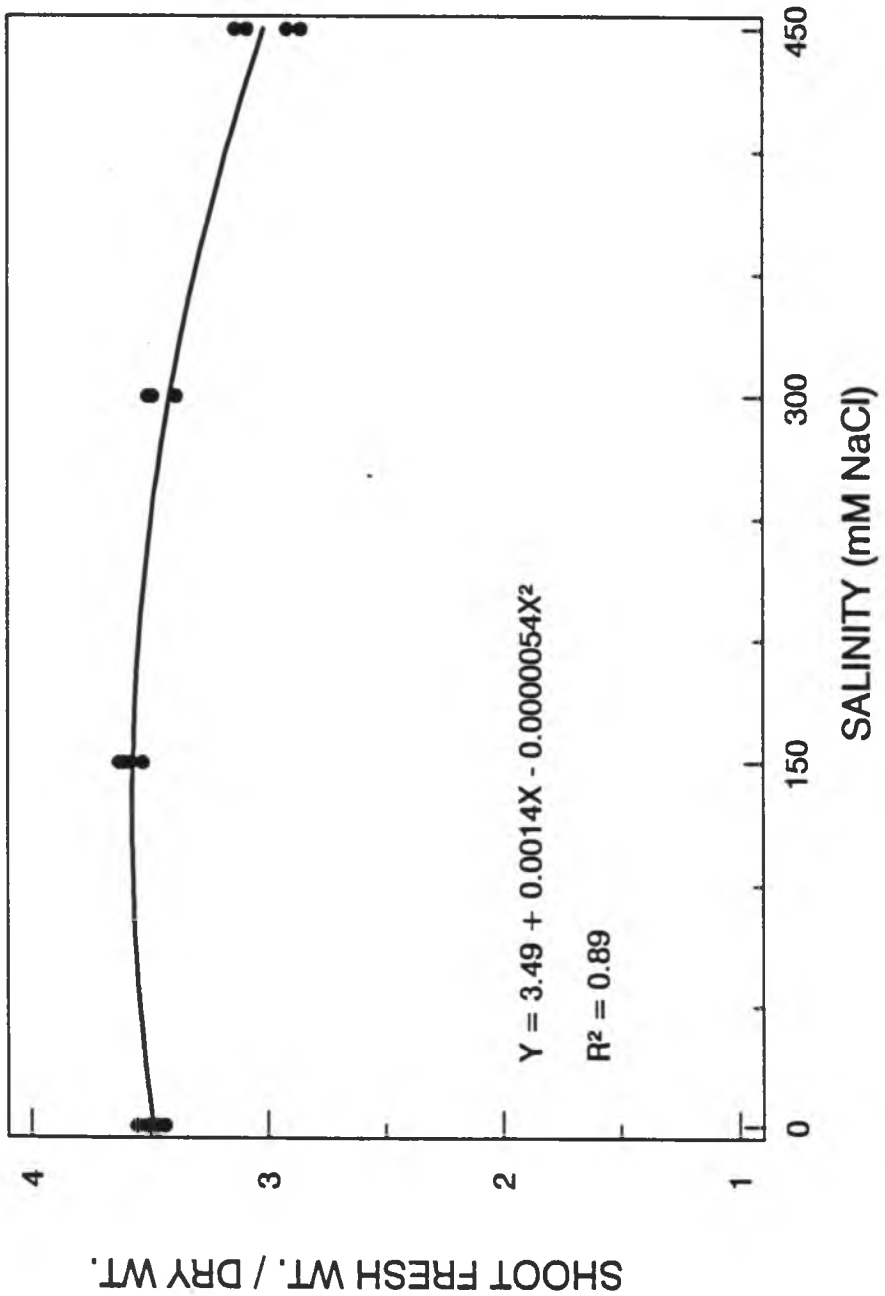


Figure 50. Shoot fresh weight/dry weight as influenced by NaCl level.

dehydration (Storey and Wyn Jones, 1978b; Sandhu *et al.*, 1981). There was a slight increase in shoot tissue succulence at 150 *mM* NaCl, which coincided with shoot growth stimulation. Increased succulence is typical of most dicotyledonous halophytes, which accumulate Na^+ and Cl^- when grown under saline conditions (Flowers and Yeo, 1986).

Shoot concentrations of Na^+, K^+, Cl^-, and total soluble carbohydrates on a whole cell tissue water basis are shown in Figure 51. Estimates were made of the contributions of the various solutes present to osmotic adjustment (Fig. 52), assuming an osmotic coefficient of 0.9 for inorganic ions, and of 1.0 for sugars (Lang, 1967).

The concentration of Na^+ in the shoot sap increased rapidly, reaching 415 *mM* at high salinity (Fig. 51). Sodium was the major contributor to osmotic adjustment at higher salinities (Fig. 52). Potassium was the major contributor to osmotic adjustment at 1 *mM* NaCl, but its role declined at higher salinity. The contribution of Cl^- to shoot osmotic adjustment also increased with increasing salinity, and Na^+ and Cl^- accounted for about 50% of the osmotic adjustment of the shoot sap at high salinity. Soluble carbohydrates also made substantial contributions to osmotic adjustment. The remainder of shoot osmotic adjustment not accounted for declined from 25 to 17%, and was probably due to amino and organic acids.

Compatible Solutes

Proline concentrations increased in shoots, particularly at salinities greater than 150 *mM* NaCl (Fig. 53). However, levels were much lower than in bermudagrass (cf. Chapter V). Both glycinebetaine

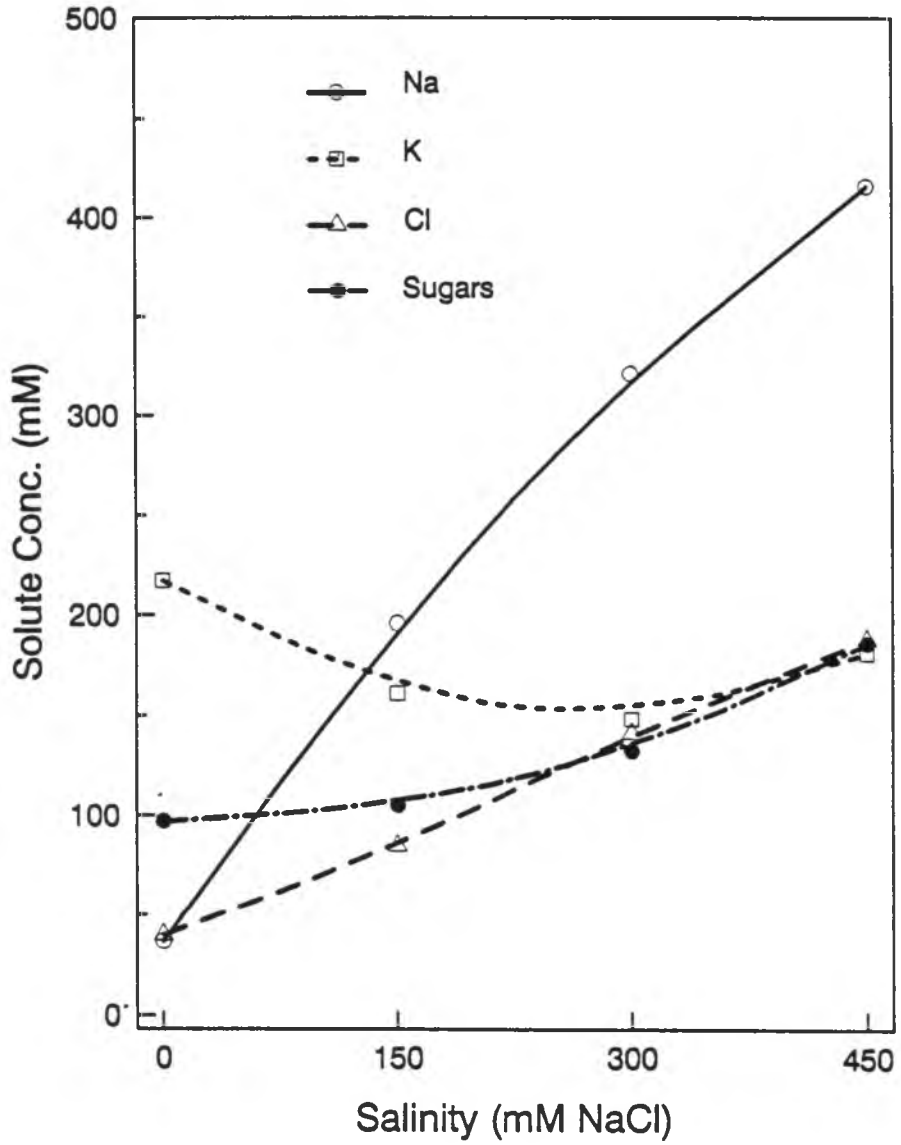


Figure 51. Shoot solute concentrations , expressed on a tissue water basis, as influenced by NaCl level.

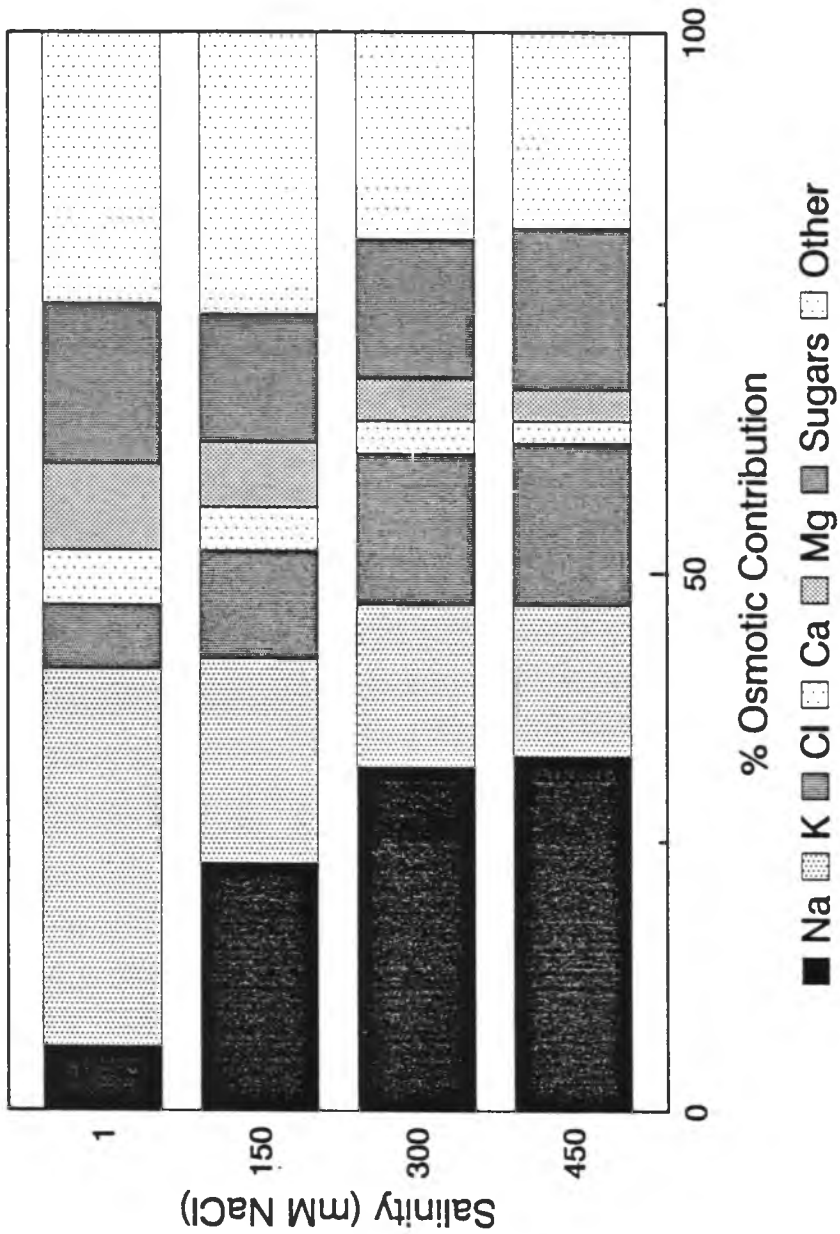


Figure 52. Calculated contributions of solutes to the osmotic adjustment of shoots at different salinities, assuming an osmotic coefficient of 0.9 for inorganic ions and 1.0 for sugars.

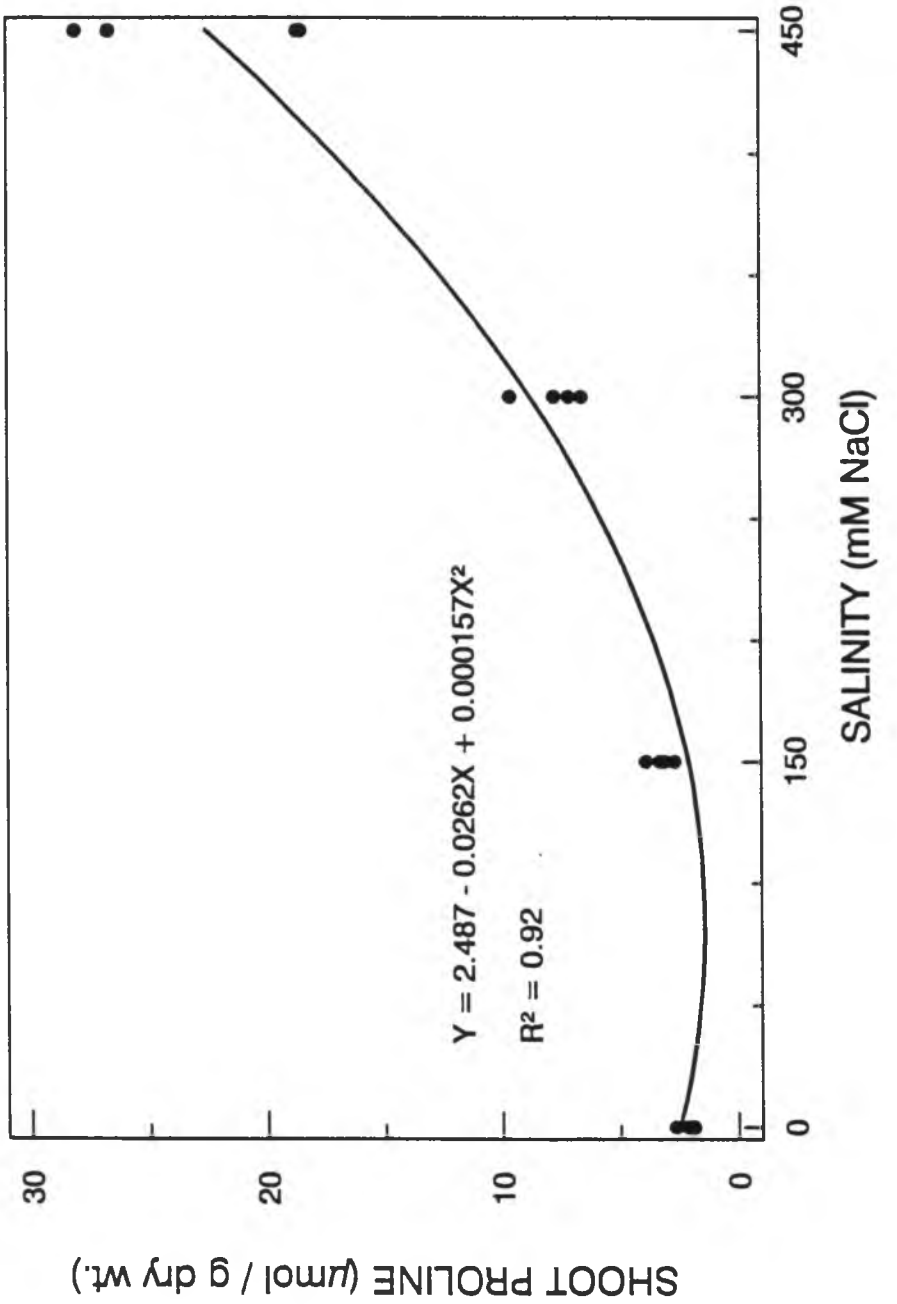


Figure 53. Shoot proline concentration, expressed on a dry weight basis, as influenced by NaCl level.

and trigonelline increased in shoots with increasing salinity, reaching levels of 257 and 0.3 μmol g^{-1} dry wt., respectively (Figs. 54-55). The concentration of trigonelline would be too low to be osmotically active, even if located exclusively in the cytoplasm.

A widely accepted hypothesis has been advanced that glycinebetaine, proline, and possibly other compounds may act as non-toxic cytoplasmic osmotica in various salt tolerant plants (Flowers *et al.*, 1977; Wyn Jones, 1984; Stewart and Lee, 1974). Preliminary estimates of the contribution of glycinebetaine and proline to cytoplasmic osmotic adjustment were made, assuming that these compounds are confined to the cytoplasm which occupies 10% of the tissue water volume (Table 12). There is substantial evidence that glycinebetaine and proline are located primarily, though probably not wholly, in the cytoplasm (Hall *et al.*, 1978; Gorham and Wyn Jones, 1983). On the basis of these assumptions, concentrations of glycinebetaine in *S. virginicus* would be adequate for osmotic adjustment of the cytoplasm above a basal osmolality of 260-440 mOsmol kg^{-1} (Wyn Jones, 1984).

DISCUSSION

A true halophyte has been defined as one in which growth is stimulated by moderate concentrations of NaCl, and by the ability to survive and grow in media having osmotic potentials of -1.5 MPa and lower (approximately 340 *mM* NaCl) (Flowers *et al.*, 1977). *Sporobolus virginicus* meets both of these criteria.

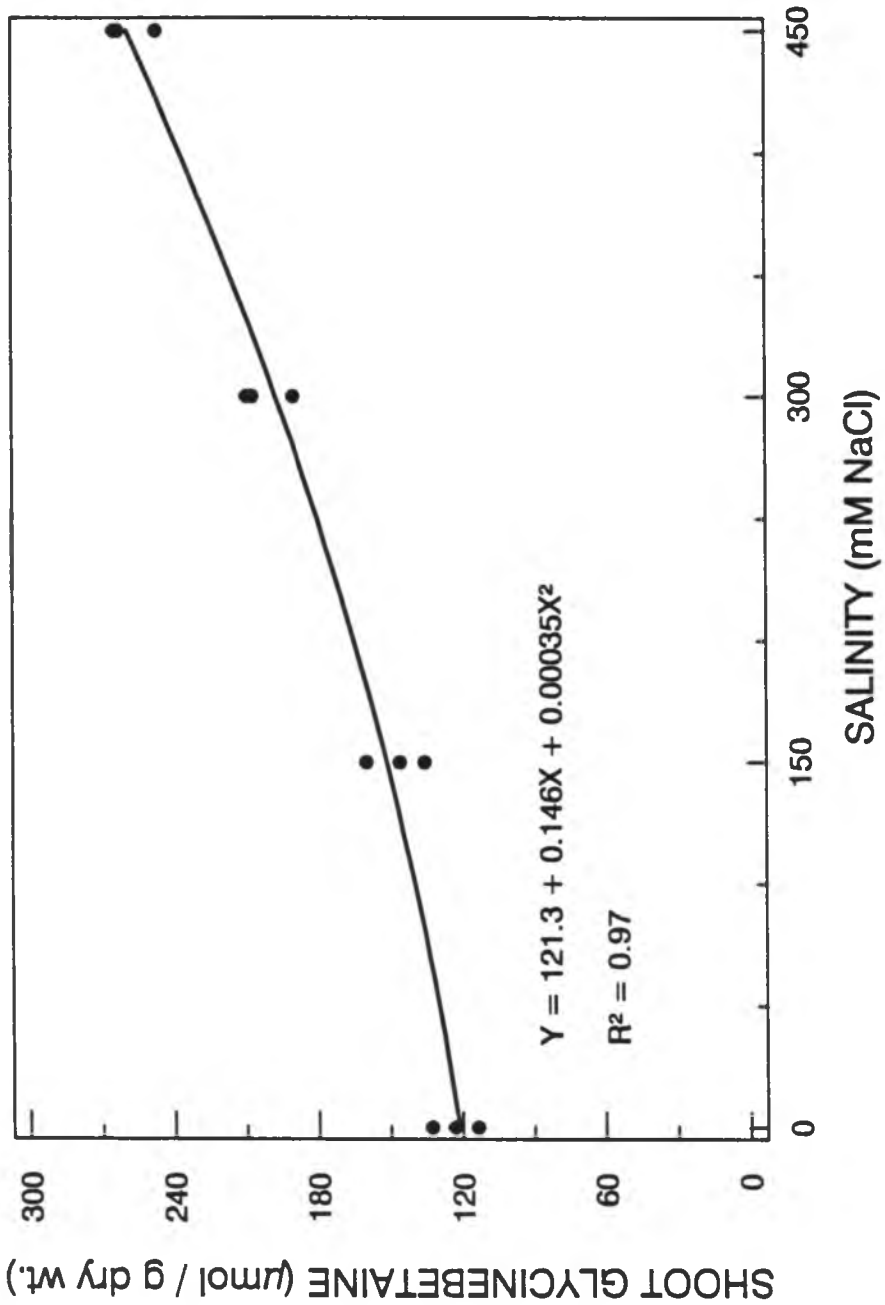


Figure 54. Shoot glycinebetaine concentration, expressed on a dry weight basis, as influenced by NaCl level.

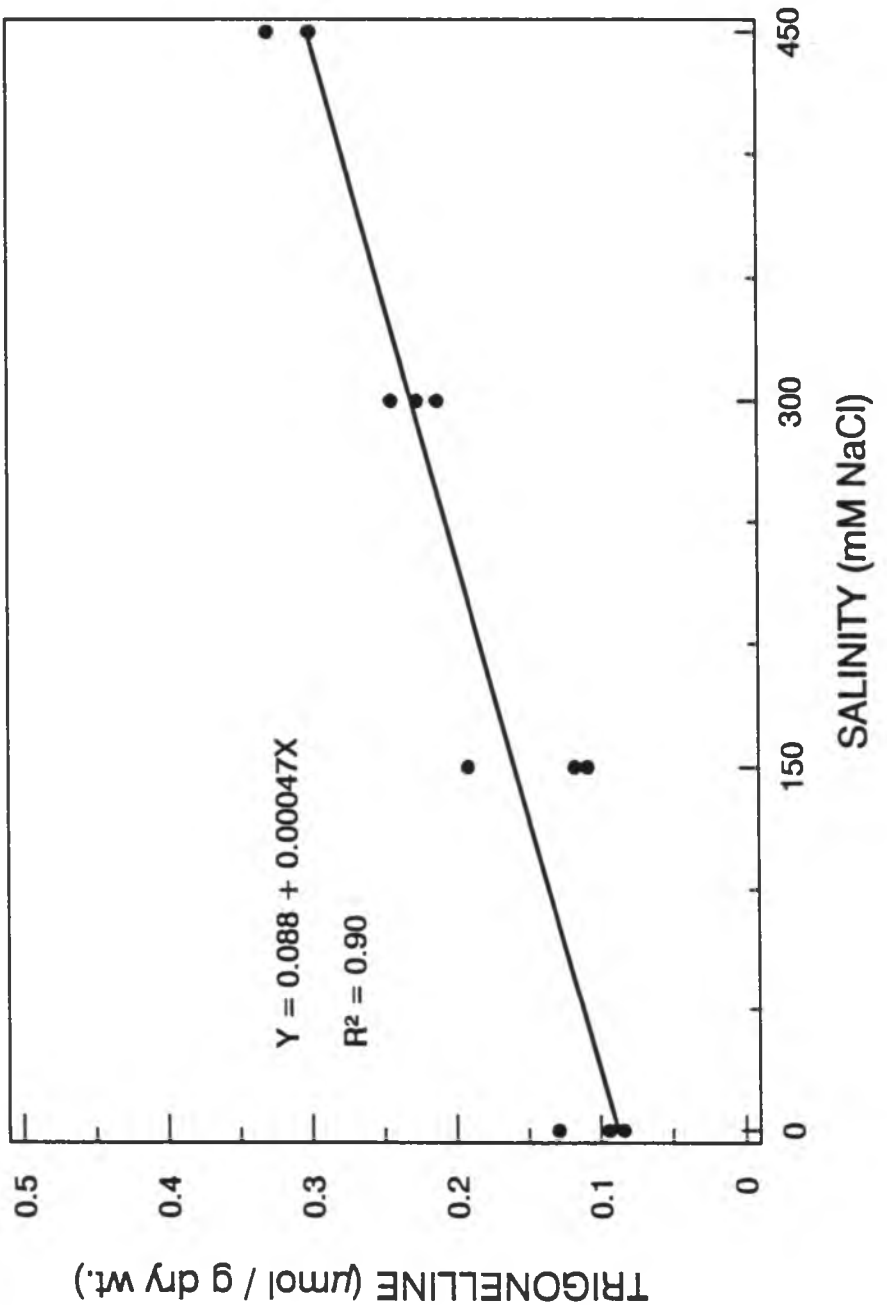


Figure 55. Shoot trigonelline concentration, expressed on a dry weight basis, as influenced by NaCl level.

Table 12. Shoot glycinebetaine and proline contents and their possible osmotic contributions to cytoplasmic osmotic adjustment.

Salinity (*mM*)	Shoot Tissue Content μmol g^{-1} drw wt. or (*mM*)[z]				[y]Estimated contribution to cytoplasmic osmotic adjustment (mOsmol kg^{-1})
	Glycinebetaine		Proline		
1	123	(50)	2.1	(0.9)	510
150	147	(58)	3.2	(1.3)	590
300	202	(82)	7.7	(3.1)	850
450	257	(126)	22.9	(11.4)	1370

[z]Tissue in brackets are concentrations calculated on a whole cell tissue water basis.

[y]Estimate assuming glycinebetaine and proline to be concentrated in cytoplasm occupying 10% of the cell volume.

Maximum shoot growth of *S. virginicus* occured at approximately 150 *mM* NaCl. This coincided with a rapid influx of Na^+ and Cl^- to the shoots at moderate salinity, and a corresponding slight increase in shoot succulence. An increase in shoot succulence and growth coinciding with shoot ion uptake is characteristic of halophytes (Flowers *et al.*, 1977). Even though Na^+ is required in trace amounts in C_4 plants (Brownwell and Crossland, 1972), the increased growth coinciding with increased shoot Na^+ concentrations is probably due to an improvement in the osmotic status of the shoot (Gale *et al.*, 1970).

Osmotic adjustment of the shoots paralleled the progressive decrease in the osmotic potential of the medium, though differences became less with increasing salinity. Increased concentrations of Na^+ and Cl^- in the shoots were responsible for osmotic adjustment at low to intermediate salinity. However, at high salinity tissue dehydration was partially responsible, though accumulation of Na^+ and Cl^- in shoots continued to occur.

It is generally held that growth inhibition associated with tissue dehydration at high salinity is due to water stress, and resultant loss of cell turgor, resulting from inadequate tissue osmotic adjustment (Radin, 1983; Hellebust, 1976). However, the supply of ions to shoots of *S. virginicus* was adequate for osmotic adjustment, as large quantities of Na^+ and Cl^- were transported to the shoots and subsequently secreted from the leaves. The causes of growth reduction in salt-stressed monocots remain elusive (Munns *et al.*, 1982; Greenway and Munns, 1983; Yeo, 1983). The use of ions for osmotic adjustment requires active transport across both plasmalemma and tonoplast. Growth

limitation at high salinity may be due to an inadequate respiratory system to provide the energy for active transport, or alternatively there may be an insufficient number of carriers required for the fast rate of ion uptake required for cell elongation under saline conditions (Yeo, 1983; Greenway and Munns, 1983). Reduced photosynthetic capacity may be a reason for reduced growth under salinity. Salinity stress reduced both stomatal conductance and photosynthetic capacity of the mesophyll in *Agrostis stolonifera* (Robertson and Wainwright, 1987). It has been proposed that at high salinities cell expansion could be reduced by a buildup of salts in cell walls (apoplast) which would effectively reduce cell turgor (Oertli, 1968; Flowers and Yeo, 1986). However, active salt glands should prevent apoplastic buildup of ions (Flowers and Yeo, 1986). Alternately, ion concentrations in the cytoplasm may build up to toxic levels at high salinity if vacuolar ion compartmentation becomes inadequate (Storey and Wyn Jones, 1979).

S. virginicus was able to limit the accumulation of Na^+ and Cl^- in the shoots to a level that did not exceed that required for osmotic adjustment. This can be seen in the uptake pattern of Na^+ and Cl^-, which increased rapidly at low salinities, but then leveled off at higher salinities (Figs. 38-39). *S. virginicus* can be grouped with halophytic species which maintain low levels of inorganic ions in the shoots, and concurrently maintain relatively low shoot tissue water levels under high salinity, a group comprised mainly of monocotyledonous halophytes (Briens and Larher, 1982; Albert and Popp, 1977; Gorham *et al.*, 1980). Sodium and Cl^- levels in shoots were regulated largely by a massive secretion from leaf salt glands. The amount of ions secreted on

a daily basis from plants growing under 450 *mM* NaCl was determined to be 4 times the amount of Na^+, and 6 times the amount of Cl^- found in the shoots. Such secretion would place a large energy demand on plants, which may contribute to growth inhibition under high salinity.

Shoots of *S. virginicus* maintained a high selectivity for K^+ at high salinity, despite a substantial secretion of K^+ salts by salt glands. The maintenance of relatively constant K^+ concentrations in shoots under high salinity has been noted in a number of other halophytes, and may be an integral part of halophytism (Wyn Jones *et al.*, 1979). The high affinity for K^+ in shoots and the maintenance of constant K^+ at high salinity may be interpreted as a requirement for a minimum cytoplasmic K^+, possibly associated with the K^+ requirement of protein synthesis (Wyn Jones et al., 1979).

Potassium concentration in roots actually increased with increasing salinity (Fig. 43). A NaCl stimulation of K^+ uptake has been reported in a number of halophytes, including *Suaeda monoica* (Story and Wyn Jones, 1979), and *Triglochin maritima* (Jefferies, 1973). This is in contrast to the situation in glycophytic plants, such as barley (Storey and Wyn Jones, 1978b), and soybean (Lauchli and Wienecke, 1979). Potassium accumulation in halophyte roots may function as a K^+ reservoir when the plant is exposed to high Na^+/low K^+ environments. Potassium accumulation in roots, coupled with selective ion secretion by leaf salt glands, are no doubt involved in maintaining a high shoot selectivity for K^+ over Na^+.

Monocotyledonous plants often accumulate soluble carbohydrates under salt stress (Briens and Larher, 1982; Gorham *et al.*, 1981). Total

soluble carbohydrates increased in shoots of *S. virginicus* to 380 μmol g^{-1} dry wt. at 450 *mM* NaCl, which is similar to levels found in the grass halophytes *Puccinellia maritima* and *Spartina townsendii* (Briens and Larher, 1982). The large amounts of soluble carbohydrates found in many grass halophytes (and in *S. virginicus*) must indicate that a substantial proportion is located in the vacuoles, though it is likely that some may contribute to cytoplasmic osmoregulation as well (Gorham *et al.*, 1980). Soluble carbohydrates contributed to whole-cell osmotic adjustment in *S. virginicus*. However, the use of sugars for osmotic adjustment on a whole-cell basis is inefficient in terms of energy, requiring ten times more energy than the transport and compartmentation of an equivalent amount (on an osmotically active basis) of ions (Gorham *et al.*, 1980). The high energy cost may have contributed to the growth reduction of *S. virginicus* observed at high salinity.

Glycinebetaine, and in some cases proline, have been proposed as compatible cytoplasmic solutes in certain salt tolerant plants (Wyn Jones, 1984; Stewart and Lee, 1974). There is evidence that these compounds are located predominantly in the cytoplasm (Wyn Jones, 1984; Gorham and Wyn Jones, 1983). The possible contributions of glycinebetaine and proline to cytoplasmic osmotic adjustment were estimated, assuming that these compounds were located in the cytoplasm, comprising 10% of the total cell volume. If the assumptions hold, the concentrations of glycinebetaine and proline would be sufficient for total osmotic adjustment of the cytoplasm (above a basal cytoplasmic osmotic potential of 350-400 mOsmol kg^{-1}) at intermediate to high

salinities. However, the significance of glycinebetaine and proline as compatible cytoplasmic solutes cannot be assessed without further direct evidence of their intracellular distribution.

CHAPTER VII

CONCLUSIONS

Growth and physiological responses to salinity of 13 C_4 turfgrasses, and a C_4 coastal salt marsh grass, were compared in these studies. The objective was to elucidate salinity tolerance mechanisms operating in these grasses. Experiments were conducted by solution culture in a glasshouse. Grasses included in these studies are listed below.

Seashore paspalum (*Paspalum vaginatum* Swartz)

St. Augustinegrass (*Stenotaphrum secundatum* Walt.)

a) cv. Floratine

b) Hawaii selection

Centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.)

Manilagrass (*Zoysia matrella* (L.) Merr.)

Japanese lawngrass (*Zoysia japonica* Steud.)

Common bermudagrass (*Cynodon dactylon* (L.) Pers.)

a) cv. FB-137

b) Hawaii selection #1

c) Hawaii selection #2

Hybrid bermudagrass (*Cynodon dactylon X C. transvaalensis* Burtt-Davey)

a) cv. Tifway

b) cv. Tifgreen

c) cv. Tifdwarf

Sunturf bermudagrass (*Cynodon magennisii* Hurcombe)

Sand dropseed (*Sporobolus virginicus* (L.) Kunth)

Relative shoot growth reduction with increasing salinity, as a percent of control, was used as an indication of salt tolerance. Seashore paspalum, the St. Augustinegrasses, and manilagrass consistently ranked as the most salt tolerant. The bermudagrasses were intermediate in salt tolerance. The common bermudagrass Hawaii selections were slightly more salt tolerant than the other bermudagrasses (Chapter III). Japanese lawngrass was salt sensitive, and centipedegrass was very salt sensitive, both grasses growing poorly under intermediate to low salinities, respectively.

The effect of salinity on turfgrass quality was evaluated in 6 turfgrasses (Chapter V). Turfgrasses were visually scored for color and live shoot density. Seashore paspalum, St. Augustinegrass, and manilagrass maintained relatively dense, green turf up to 400 *mM* NaCl, continuing to produce new shoots, though at a slow rate. Quality dropped to a much lower level at high salinity in bermudagrass. Quality rapidly declined at low salinities in Japanese lawngrass and centipedegrass, with total shoot death occuring at intermediate salinities.

Root growth increased under intermediate salinities, then decreased, in the bermudagrasses, manilagrass, and seashore paspalum, the tendency being greatest in seashore paspalum (Chapter III). Root growth of the other turfgrasses declined with increasing salinity. Root growth of *Sporobolus virginicus* increased linearly with increasing salinity to 450 *mM* NaCl. Increased root growth may be an adaptation to

salinity, resulting in more efficient water and nutrient uptake (Gorham *et al.*, 1985b).

All grasses adjusted osmotically, maintaining their shoot sap osmolalities above that of the growing solutions. Osmotic adjustment was accomplished primarily by an increase in shoot Na^+ and Cl^+ concentrations. However, shoot dehydration occured with increasing salinity in all turfgrasses. Shoot fresh wt./dry wt. (shoot succulence) increased in *Sporobolus virginicus* under intermediate salinity, then declined. The concentrating effect of tissue dehydration contributed to shoot osmotic adjustment.

Stimulation of shoot growth occured at 100 to 150 *mM* NaCl in *Sporobolus virginicus* and St. Augustinegrasses. Growth stimulation was concurrent with a rapid increase in shoot Na^+ and Cl^+ in both grasses, and with an increase in shoot succulence in *S. virginicus*. Stimulation of shoot growth coinciding with rapid increases in shoot ion concentrations, and frequently of shoot succulence, is characteristic of many halophytes (Flowers *et al.*, 1977). Even though Na^+ is required in C_4 plants (Brownwell and Crossland, 1972), the increased growth coinciding with increased shoot Na^+ and Cl^- is probably due to an improvement in the osmotic status of the shoot (Gale *et al.*, 1970).

Salinity tolerance among closely related grasses has been associated with exclusion of Na^+ salts from shoots (Gorham *et al.*, 1985b). Seashore paspalum, manilagrass, and bermudagrasses maintained shoot Na^+ and Cl^- at relatively low levels under high salinity. (In Chapter III shoot Na^+ and Cl^- levels of seashore paspalum were intermediate at high salinity). *S. virginicus* was also able to strictly

limit accumulation of Na^+ and Cl^- in shoots at high salinities. Only about 50% of leaf sap osmolality was due to Na^+ and Cl^- at 450 *mM* NaCl. Soluble carbohydrates substantially contributed to the remainder of shoot osmotic adjustment on a whole cell basis. In contrast, shoot Na^+ and Cl^- levels were relatively high in centipedegrass and Japanese lawngrass, particularly if expressed on a tissue water concentration basis. Na^+ and Cl^- toxicity was evident in centipedegrass and Japanese lawngrass, which suffered severe leaf burn and shoot dieback at low to intermediate salinities.

A high shoot selectivity for K^+ was evident in seashore paspalum and manilagrass (Chapter V) and in bermudagrasses (Chapter III). Shoot selectivity for K^+ may be involved in maintaining minimum basal K^+ shoot levels which are required in the cytoplasm for translation and other processes (Wyn Jones, 1984). Active ion discrimination (K^+ uptake/Na^+ exclusion) occurs at the root cortex through selective K^+ uptake/Na^+ exclusion at the plasmalemma or K/Na^+ exchange at the tonoplast. Alternately, in some salt tolerant plants shoot salt exclusion occurs by secretion from leaf salt glands. Seashore paspalum relied exclusively on K^+/Na^+ selectivity of the root cortex to maintain Na^+ exclusion from shoots. However, *S. virginicus*, manilagrass, and bermudagrasses were also able to limit shoot Na^+ and Cl^- by the presence of highly active leaf salt glands. Secretion was selective for Na^+ and Cl^- over K^+, Ca^{2+}, and Mg^{2+}, although K^+ secretion did occur, particularly in *S. virginicus*. Though salt glands were present in Japanese lawngrass, they were much less efficient, secreting only half the Na^+ and Cl^- per leaf weight as manilagrass and bermudagrass. As a consequence, Japanese

lawngrass did not limit shoot Na^+ and Cl^- as effectively, resulting in high shoot ion levels at intermediate salinities.

St. Augustinegrass responded differently to salinity than did the other grasses, and was in many ways similar to the highly salt tolerant dicotyledonous halophytes. Shoot growth was stimulated at intermediate salinities, concurrent with large increases in shoot Na^+ and Cl^- concentrations and high tissue succulence. Salt accumulation in shoots is generally associated with tissue succulence and growth stimulation in dicotyledonous halophytes, due to efficient compartmentation of salts in large vacuoles of mesophyll cells, coupled with osmotic influx of water (Kramer, 1984). The high Na^+ and Cl^- levels, and the very low K^+/Na^+ ratios in shoots were similar in magnitude to dicotyledonous halophytes of the families Chenopodiaceae and Caryophyllaceae (Gorham *et al.*, 1980).

When tissue concentration of NaCl exceeds about 200 *mM* (as did occur in all grasses in this study at high salinity) ion compartmentation within the vacuole becomes necessary to avoid enzyme deactivation and subsequent cell death. Under these conditions, the maintenance of osmotic equilibrium across the tonoplast requires the accumulation in the cytoplasm of nontoxic "compatible solutes", the most likely candidates in the Poaceae being glycinebetaine and proline (Gorham *et al.*, 1985b; Wyn Jones, 1981).

There is substantial evidence that the location of glycinebetaine and proline is predominately in the cytoplasm. The relative contributions of glycinebetaine and proline to the osmotic adjustment of

the cytoplasm were estimated, with the assumptions that they were located within a cytoplasmic volume comprising 10% of the cell volume.

On the basis of these assumptions, cytoplasmic concentrations of glycinebetaine and proline would be adequate for complete (or almost complete) cytoplasmic osmotic adaptation in all grasses except centipedegrass, accomodating all the increase in cytoplasmic osmotic pressure above a basal level of 300-400 mOsmol kg^{-1}. Glycinebetaine and proline levels in centipedegrass were too low to affect any significant osmotic adjustment of the cytoplasm. This lack of compatible solutes may mean that centipedegrass was unable to compartmentalize ions within shoot vacuoles. This, coupled with high Na^+ and Cl^- shoot concentrations due to an inability to restrict these ions from the shoots, may be responsible for the salt sensitivity of centipedegrass.

APPENDIX A

(CHAPTER III)

Table 13. Analysis of variance for regressing root dry weight on salinity across grasses.

Dependent Variable: Root Dry Weight

Source	DF	Sum of Squares	F Value	Pr > F
Model	32	9.7913	8.55	0.0001
Salinity	1	0.9128	5.38	0.0224
Sal.xSal.	1	0.4216	11.77	0.0009
Grass	10	6.5987	18.43	0.0001
Sal.xGrass	10	2.0242	5.65	0.0001
Sal.xSal.xGrass	10	0.5540	1.55	0.1341
Error	99	3.5447		

Table 14. Analysis of variance for regressing shoot Na^+ on salinity across grasses.

Dependent Variable: Shoot Na^+

Source	DF	Sum of Squares	F Value	Pr > F
Model	32	48.04	131.20	0.0001
Salinity	1	8.23	719.56	0.0001
Sal.xSal.	1	1.58	138.35	0.0001
Grass	10	32.43	283.51	0.0001
Sal.xGrass	10	3.91	34.17	0.0001
Sal.xSal.xGrass	10	1.88	16.44	0.0001
Error	99	1.13		

Table 15. Analysis of variance for regressing shoot Cl^- on salinity across grasses.

Dependent Variable: Shoot Cl^-

Source	DF	Sum of Squares	F Value	Pr > F
Model	32	15.94	73.79	0.0001
Salinity	1	4.28	633.58	0.0001
Sal.xSal.	1	0.49	73.03	0.0001
Grass	10	8.79	130.02	0.0001
Sal.xGrass	10	2.02	29.95	0.0001
Sal.xSal.xGrass	10	0.359	5.32	0.0001
Error	99	0.67		

Table 16. Analysis of variance for regressing root Na^+ on salinity across grasses.

Dependent Variable: Root Na^+

Source	DF	Sum of Squares	F Value	Pr > F
Model	32	14.83	117.73	0.0001
Salinity	1	9.56	2428.78	0.0001
Sal.xSal.	1	0.09	22.41	0.0001
Grass	10	3.99	101.31	0.0001
Sal.xGrass	10	0.88	22.44	0.0001
Sal.xSal.xGrass	10	0.31	7.87	0.0001
Error	99	15.22		

Table 17. Analysis of variance for regressing root Cl^- on salinity across grasses.

Dependent Variable: Root Cl^-

Source	DF	Sum of Squares	F Value	Pr > F
Model	32	4.589	100.29	0.0001
Salinity	1	2.579	1804.13	0.0001
Sal.xSal.	1	0.002	1.13	0.2925
Grass	10	1.440	100.74	0.0001
Sal.xGrass	10	0.488	34.15	0.0001
Sal.xSal.xGrass	10	0.079	5.53	0.0001
Error	99	0.079		

Table 18. Analysis of variance for regressing shoot K^+ on salinity across grasses.

Dependent Variable: Shoot K^+

Source	DF	Sum of Squares	F Value	Pr > F
Model	32	8.870	35.04	0.0001
Salinity	1	1.752	221.51	0.0001
Sal.xSal.	1	0.433	54.76	0.0001
Grass	10	4.658	58.89	0.0001
Sal.xGrass	10	1.335	16.88	0.0001
Sal.xSal.xGrass	10	0.692	8.75	0.0001
Error	99	0.783		

Table 19. Analysis of variance for regressing root K^+ on salinity across grasses.

Dependent Variable: Root K^+

Source	DF	Sum of Squares	F Value	Pr > F
Model	32	2.100	41.79	0.0001
Salinity	1	0.189	120.26	0.0001
Sal.xSal.	1	0.000	0.01	0.9110
Grass	10	1.514	96.41	0.0001
Sal.xGrass	10	0.341	21.72	0.0001
Sal.xSal.xGrass	10	0.056	3.57	0.0004
Error	99	0.155		

Table 20. Analysis of variance for regressing leaf sap osmolality on salinity across grasses.

Dependent Variable: Leaf Sap Osmolality

Source	DF	Sum of Squares	F Value	Pr > F
Model	32	3821172	21.48	0.0001
Salinity	1	2542126	457.27	0.0001
Sal.xSal.	1	15254	2.74	0.1008
Grass	10	761635	13.70	0.0001
Sal.xGrass	10	336575	6.05	0.0001
Sal.xSal.xGrass	10	165581	2.98	0.0026
Error	99			

Table 21. Analysis of variance for regressing shoot tissue water content on salinity across grasses.

Dependent Variable: Shoot Tissue Water Content

Source	DF	Sum of Squares	F Value	Pr > F
Model	21	309.85	166.33	0.0001
Salinity	1	21.72	244.93	0.0001
Grass	10	277.52	312.85	0.0001
Sal.xGrass	10	10.60	11.95	0.0001
Error	110	9.76		

APPENDIX B
(CHAPTER V)

Table 22. Analysis of variance for regressing shoot growth rate on salinity across grasses.

Dependent Variable: Shoot Growth Rate

Source	DF	Sum of Squares	F Value	Pr > F
Model	17	13.146	80.94	0.0001
Salinity	1	2.980	311.89	0.0001
Sal.xSal.	1	0.113	11.87	0.0008
Grass	5	8.911	186.55	0.0001
Sal.xGrass	5	0.267	5.59	0.0001
Sal.xSal.xGrass	5	0.875	18.32	0.0001
Error	132	1.261		

Table 23. Analysis of variance for regressing relative shoot growth on salinity across grasses.

Dependent Variable: Relative Shoot Growth

Source	DF	Sum of Squares	F Value	Pr > F
Model	17	266521	69.30	0.0001
Salinity	1	110139	486.83	0.0001
Sal.xSal.	1	1299	5.74	0.0179
Grass	5	120528	106.55	0.0001
Sal.xGrass	5	8479	7.50	0.0001
Sal.xSal.xGrass	5	26074	23.05	0.0001
Error	132	29863		

Table 24. Analysis of variance for regressing shoot visual quality rating on salinity across grasses.

Dependent Variable: Shoot Visual Quality Rating

Source	DF	Sum of Squares	F Value	Pr > F
Model	17	889.11	76.33	0.0001
Salinity	1	347.76	507.57	0.0001
Sal.xSal.	1	28.29	41.29	0.0001
Grass	5	420.55	122.76	0.0001
Sal.xGrass	5	31.80	9.26	0.0001
Sal.xSal.xGrass	5	60.71	17.72	0.0001
Error	132	90.44		

Table 25. Analysis of variance for regressing shoot fresh weight/dry weight on salinity across grasses.

Dependent Variable: Shoot Fresh Weight/Dry weight

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	230.07	733.69	0.0001
Salinity	1	32.21	1129.80	0.0001
Sal.xSal.	1	0.21	7.46	0.0076
Grass	3	190.02	2221.87	0.0001
Sal.xGrass	3	7.43	86.89	0.0001
Sal.xSal.xGrass	3	0.20	2.36	0.0773
Error	87	2.48		

Table 26. Analysis of variance for regressing leaf sap osmolality on salinity across grasses.

Dependent Variable: Leaf Sap Osmolality

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	5993084	101.24	0.0001
Salinity	1	4815856	894.92	0.0001
Sal.xSal.	1	.2797	0.52	0.4728
Grass	3	864610	53.56	0.0001
Sal.xGrass	3	294943	18.27	0.0001
Sal.xSal.xGrass	3	14876	0.92	0.4339
Error	99	6466640		

Table 27. Analysis of variance for regressing shoot Na^+ on salinity across grasses.

Dependent Variable: Shoot Na^+

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	9.124	157.12	0.0001
Salinity	1	2.666	504.98	0.0001
Sal.xSal.	1	0.221	41.84	0.0001
Grass	3	5.834	368.37	0.0001
Sal.xGrass	3	0.311	19.65	0.0001
Sal.xSal.xGrass	3	0.091	5.79	0.0033
Error	28	0.148		

Table 28. Analysis of variance for regressing shoot Cl^- on salinity across grasses.

Dependent Variable: Shoot Cl^-

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	1.406	180.19	0.0001
Salinity	1	0.435	612.92	0.0001
Sal.xSal.	1	0.004	5.75	0.0234
Grass	3	0.784	368.49	0.0001
Sal.xGrass	3	0.178	83.75	0.0001
Sal.xSal.xGrass	3	0.005	2.23	0.1055
Error	39	1.426		

Table 29. Analysis of variance for regressing shoot Na^+ (on a tissue water basis) on salinity across grasses.

Dependent Variable: Shoot Na^+

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	620790	79.97	0.0001
Salinity	1	496700	703.81	0.0001
Sal.xSal.	1	5012	7.10	0.0126
Grass	3	92109	43.51	0.0001
Sal.xGrass	3	25712	12.14	0.0001
Sal.xSal.xGrass	3	1256	0.59	0.6247
Error	28	19760		

Table 30. Analysis of variance for regressing on shoot Cl^- (on a tissue basis) salinity across grasses.

Dependent Variable: Shoot Cl^-

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	97443	70.88	0.0001
Salinity	1	68253	546.15	0.0001
Sal.xSal.	1	70	0.56	0.4594
Grass	3	15394	41.06	0.0001
Sal.xGrass	3	13570	36.20	0.0001
Sal.xSal.xGrass	3	154	0.41	0.7448
Error	28	3499		

Table 31. Analysis of variance for regressing shoot K^+ on salinity across grasses.

Dependent Variable: Shoot K^+

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	2.030	84.76	0.0001
Salinity	1	0.517	237.63	0.0001
Sal.xSal.	1	0.140	64.17	0.0001
Grass	3	1.226	187.71	0.0001
Sal.xGrass	3	0.094	14.41	0.0001
Sal.xSal.xGrass	3	0.053	8.09	0.0005
Error	28	0.061		

Table 32. Analysis of variance for regressing on shoot Ca^{2+} salinity across grasses.

Dependent Variable: Shoot Ca^{2+}

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	0.070	72.74	0.0001
Salinity	1	0.023	267.36	0.0001
Sal.xSal.	1	0.004	44.46	0.0001
Grass	3	0.038	144.88	0.0001
Sal.xGrass	3	0.003	13.37	0.0001
Sal.xSal.xGrass	3	0.001	4.54	0.0103
Error	28	0.002		

Table 33. Analysis of variance for regressing shoot Mg^{2+} on salinity across grasses.

Dependent Variable: Shoot Mg^{2+}

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	0.0488	88.82	0.0001
Salinity	1	0.0238	475.33	0.0001
Sal.xSal.	1	0.0030	61.10	0.0001
Grass	3	0.0163	108.88	0.0001
Sal.xGrass	3	0.0046	31.09	0.0001
Sal.xSal.xGrass	3	0.0010	6.89	0.0013
Error	39	0.0502		

Table 34. Analysis of variance for regressing root Na^+ on salinity across grasses.

Dependent Variable: Root Na^+

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	7.927	41.05	0.0001
Salinity	1	5.299	301.89	0.0001
Sal.xSal.	1	0.275	15.68	0.0005
Grass	3	1.706	32.40	0.0001
Sal.xGrass	3	0.523	9.93	0.0001
Sal.xSal.xGrass	3	0.123	2.34	0.0950
Error	28	0.491		

Table 35. Analysis of variance for regressing root Cl^- on salinity across grasses.

Dependent Variable: Root Cl^-

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	3.442	110.25	0.0001
Salinity	1	2.125	748.96	0.0001
Sal.xSal.	1	0.032	11.25	0.0023
Grass	3	0.851	99.94	0.0001
Sal.xGrass	3	0.417	48.99	0.0001
Sal.xSal.xGrass	3	0.016	1.92	0.1486
Error	28	0.079		

Table 36. Analysis of variance for regressing root K^+ on salinity across grasses.

Dependent Variable: Root K^+

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	0.3064	26.89	0.0001
Salinity	1	0.0108	10.46	0.0031
Sal.xSal.	1	0.0002	0.22	0.6442
Grass	3	0.2853	91.83	0.0001
Sal.xGrass	3	0.0083	2.69	0.0654
Sal.xSal.xGrass	3	0.0017	0.54	0.6608
Error	28	0.0290		

Table 37. Analysis of variance for regressing root Ca^{2+} on salinity across grasses.

Dependent Variable: Root Ca^{2+}

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	0.0150	27.96	0.0001
Salinity	1	0.0119	243.66	0.0001
Sal.xSal.	1	0.0007	15.15	0.0006
Grass	3	0.0013	9.07	0.0002
Sal.xGrass	3	0.0007	4.98	0.0068
Sal.xSal.xGrass	3	0.0003	2.22	0.1083
Error	39	0.0164		

Table 38. Analysis of variance for regressing root Mg^{2+} on salinity across grasses.

Dependent Variable: Root Mg^{2+}

Source	DF	Sum of Squares	F Value	Pr > F
Model	11	0.027908	41.36	0.0001
Salinity	1	0.000125	2.04	0.1645
Sal.xSal.	1	0.000000	0.01	0.9428
Grass	3	0.025415	138.12	0.0001
Sal.xGrass	3	0.002241	12.18	0.0001
Sal.xSal.xGrass	3	0.000127	0.69	0.5651
Error	39	0.029626		

Table 39. Analysis of variance for regressing shoot proline on salinity across grasses.

Dependent Variable: Shoot Proline

Source	DF	Sum of Squares	F Value	Pr > F
Model	14	159561	87.50	0.0001
Salinity	1	45295	347.77	0.0001
Sal.xSal.	1	2242	17.22	0.0001
Grass	3	67808	130.16	0.0001
Sal.xGrass	3	43097	82.72	0.0001
Sal.xSal.xGrass	3	1116	2.14	0.0878
Error	55	7163		

Table 40. Analysis of variance for regressing shoot glycinebetaine on salinity across grasses.

Dependent Variable: Shoot Glycinebetaine

Source	DF	Sum of Squares	F Value	Pr > F
Model	14	116410	35.05	0.0001
Salinity	1	66506	280.34	0.0001
Sal.xSal.	1	6439	27.14	0.0001
Grass	3	26972	28.42	0.0001
Sal.xGrass	3	13167	13.88	0.0001
Sal.xSal.xGrass	3	3324	3.50	0.0124
Error	59	13996		

Table 41. Analysis of variance for regressing shoot trigonelline on salinity across grasses.

Dependent Variable: Shoot Trigonelline

Source	DF	Sum of Squares	F Value	Pr > F
Model	14	2.8309	24.59	0.0001
Salinity	1	0.0320	3.89	0.0534
Sal.xSal.	1	0.0015	0.18	0.6709
Grass	3	2.6770	81.37	0.0001
Sal.xGrass	3	0.1092	3.32	0.0161
Sal.xSal.xGrass	3	0.0113	0.34	0.8470
Error	59	0.4852		

APPENDIX C

(CHAPTER VI)

Table 42. Analysis of variance for regressing shoot growth rate on salinity.

Dependent Variable: Shoot Growth Rate

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	0.7567	8.03	0.0054
Salinity	1	0.3436	7.29	0.0182
Sal.xSal.	1	0.4131	8.77	0.0110
Error	13	0.6124		

Table 43. Analysis of variance for regressing root dry weight on salinity.

Dependent Variable: Root Dry Weight

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	3.6261	19.50	0.0001
Salinity	1	3.5955	38.67	0.0001
Sal.xSal.	1	0.0306	0.33	0.5758
Error	13	1.2086		

Table 44. Analysis of variance for regressing shoot Na^+ on salinity.

Dependent Variable: Shoot Na^+

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	1.0832	503.08	0.0001
Salinity	1	0.9956	924.84	0.0001
Sal.xSal.	1	0.0875	81.33	0.0001
Error	9	0.0096		

Table 45. Analysis of variance for regressing shoot Cl^- on salinity.

Dependent Variable: Shoot Cl^-

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	0.1505	239.87	0.0001
Salinity	1	0.1460	465.44	0.0001
Sal.xSal.	1	0.0045	14.30	0.0043
Error	9	0.0028		

Table 46. Analysis of variance for regressing root Na^+ on salinity.

Dependent Variable: Root Na^+

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	0.6032	68.24	0.0002
Salinity	1	0.5993	135.59	0.0001
Sal.xSal.	1	0.0039	0.90	0.3873
Error	5	0.0221		

Table 47. Analysis of variance for regressing root Cl^- on salinity.

Dependent Variable: Root Cl^-

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	0.4872	269.29	0.0001
Salinity	1	0.4763	526.55	0.0001
Sal.xSal.	1	0.0109	12.03	0.0179
Error	5	0.0045		

Table 48. Analysis of variance for regressing shoot K^+ on salinity.

Dependent Variable: Shoot K^+

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	0.0597	27.24	0.0002
Salinity	1	0.0451	41.19	0.0001
Sal.xSal.	1	0.0146	13.28	0.0054
Error	9	0.0099		

Table 49. Analysis of variance for regressing root K^+ on salinity.

Dependent Variable: Root K^+

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	0.0399	21.73	0.0034
Salinity	1	0.0397	43.31	0.0012
Sal.xSal.	1	0.0001	0.15	0.7160
Error	5	0.0046		

Table 50. Analysis of variance for regressing shoot Ca^{2+} on salinity.

Dependent Variable: Shoot Ca^{2+}

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	0.0032	10.87	0.0040
Salinity	1	0.0016	11.10	0.0088
Sal.xSal.	1	0.0016	10.63	0.0098
Error	9	0.0013		

Table 51. Analysis of variance for regressing shoot Mg^{2+} on salinity.

Dependent Variable: Shoot Mg^{2+}

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	0.0064	132.33	0.0001
Salinity	1	0.0058	240.90	0.0001
Sal.xSal.	1	0.0006	23.76	0.0009
Error	9	0.0002		

Table 52. Analysis of variance for regressing root Ca^{2+} on salinity.

Dependent Variable: Root Ca^{2+}

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	0.00677	33.75	0.0012
Salinity	1	0.00581	57.86	0.0006
Sal.xSal.	1	0.00097	9.64	0.0267
Error	5	0.00050		

Table 53. Analysis of variance for regressing root Mg^{2+} on salinity.

Dependent Variable: Root Mg^{2+}

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	0.00019	13.10	0.0103
Salinity	1	0.00017	23.77	0.0046
Sal.xSal.	1	0.00002	2.43	0.1801
Error	5	0.00004		

Table 54. Analysis of variance for regressing shoot soluble carbohydrates on salinity.

Dependent Variable: Shoot Soluble Carbohydrates

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	35951	17.83	0.0007
Salinity	1	35196	34.90	0.0002
Sal.xSal.	1	755	0.75	0.4093
Error	9	9076		

Table 55. Analysis of variance for regressing leaf sap osmolality on salinity.

Dependent Variable: Leaf Sap Osmolality

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	841720	222.53	0.0001
Salinity	1	839270	443.76	0.0001
Sal.xSal.	1	2450	1.30	0.2756
Error	13	24586		

Table 56. Analysis of variance for regressing shoot fresh weight/dry weight on salinity.

Dependent Variable: Shoot Fresh Weight/Dry Weight

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	0.7305	53.00	0.0001
Salinity	1	0.4977	72.22	0.0001
Sal.xSal.	1	0.2328	33.78	0.0001
Error	13	0.0896		

Table 57. Analysis of variance for regressing shoot proline on salinity.

Dependent Variable: Shoot Proline

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	1093	74.51	0.0001
Salinity	1	893	121.74	0.0001
Sal.xSal.	1	200	27.28	0.0002
Error	13	95		

Table 58. Analysis of variance for regressing shoot glycinebetaine on salinity.

Dependent Variable: Shoot Glycinebetaine

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	32314	140.26	0.0001
Salinity	1	31551	273.90	0.0001
Sal.xSal.	1	763	6.63	0.0300
Error	9	1037		

Table 59. Analysis of variance for regressing shoot trigonelline on salinity.

Dependent Variable: Shoot Trigonelline

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	0.07711	50.41	0.0001
Salinity	1	0.07554	98.78	0.0001
Sal.xSal.	1	0.00156	2.05	0.1865
Error	9	0.00688		

APPENDIX D

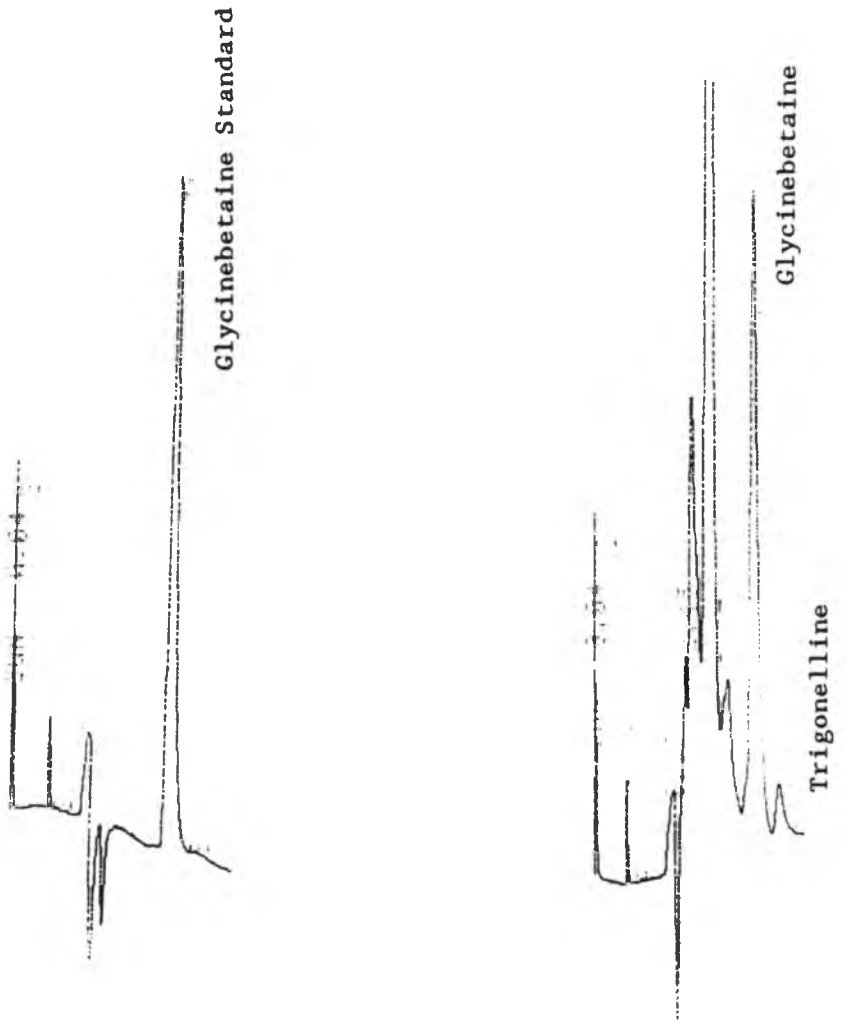
Table 60. Recovery trials of glycinebetaine added to samples of bermudagrass grown under low salinity.

A) Glycinebetaine recovered from 0.1 g samples of bermudagrass.

µg/20µL
4.68
3.95
3.56
4.69
mean=4.22

B) Percent recovery of glycinebetaine recovered from 0.1 g samples of bermudagrass to which was added 4.69 µg/20µL glycinebetaine.

Total Recovery µg/20µL	Added Glybet. Recovered µg/20µL	% Recovery
8.22	4.00	85
8.53	4.31	92
8.41	4.19	89
8.82	4.60	98
8.15	3.93	84
		mean=90%



A)2.5μg/20μL Glybet. Standard B)Grass Sample Extract

Figure 56. Representative HPLC chromatograms for glycinebetaine. A) Glycinebetaine standard. B) Grass sample extract.

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